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# NOTES ON THE ANATOMY OF ARACHNIDS

B. H. BUXTON

EIGHT DIAGRAMS AND THREE PLATES

## PART I

### THE COXAL GLANDS OF THE ARACHNIDS

This paper is a continuation and extension of one published in the *Zoologische Jahrbücher* of July, 1913, since which time I have had an opportunity of adding to the number of species examined.

It was shown in the previous paper that there are two very distinct groups of the arachnids as regards the construction of the coxal glands. In Group I may be placed the scorpions, pedipalps and spiders, which have been more particularly studied. The Phalangids certainly, and the Pseudoscorpions most probably, also belong to this group, but as they have not been made a subject of special study, will not be described in detail. In Group II were placed the Solifugae, and to these can now be added the Palpigrades.

The component parts of the coxal glands of Group I consist of (1) the terminal sac, or saccule, lined with a very delicate cubical or flattened epithelium which has the property of excreting solid particles such as carmine when injected subcutaneously. Solid particles, probably urates, can also frequently be observed normally in the cells lining the walls of the saccule. From (2) the saccule, a short duct, or collecting tubule, leads into (3) the labyrinth, which consists of a single, usually coiled, tube, the walls of which are lined with excretory epithelium having the usual striated base indicative of excretory functions, but solid particles are never excreted by these cells. At the distal end of the labyrinth there is sometimes a vesicular swelling which can be regarded as (4) the bladder; and from the

bladder (5), a short exit tubule lined with cells derived from the hypodermis leads to (6) the outlet<sup>1</sup> on the external surface, either on segments III or V, or on both of these segments, just posterior to the appendage.

In Group II the same arrangement holds good except that between the collecting tubule and the labyrinth there is a large sac lined with secretory epithelium, and the outlet is on segment II.

It was pointed out in the previous article that the coxal glands of Group I appear to be homologous with the large nephridia on segments VI and VII of *Peripatus*, whereas the coxal glands of Group II show homology with the salivary gland of *Peripatus*, the outlet of which is situated on segment II.

#### GROUP I. I. SCORPIONS AND SPIDERS

Several genera, both of scorpions and of Theraphosid spiders from the East Indies, have been recently examined, but there is nothing further to add to the description which has already been published of the coxal glands of certain American, European and African genera.

It may be recalled that the coxal gland of the scorpions lies in segments V and VI with an outlet at the base of the posterior surface of appendage V.

The coxal glands of the spiders show considerable differences. That of the Theraphosid spiders has two saccules and two outlets, one of each on segment III and the other on segment V with a large and complicated labyrinth apparently common to both saccules and outlets. The *Araneae verae* have lost the coxal gland of segment V, retaining only that of segment III; and, with the exception of the six-eyed spiders, the *Sicariids* and *Dysderids* (to which can now be added the *Oonopids*), the coxal glands of the higher spiders show various stages of degeneration, or perhaps simplification.

<sup>1</sup> There is always an outlet on the external surface, both in immature and adult specimens. The arrangement of special muscle fibers around the exit tubule indicates that it can be closed or opened at will.

## II. PEDIPALPS

Pocock has divided the Pedipalps into two distinct orders, the Uropygi and the Amblypygi. The former group consists of the Thelyphonides and Tartarides, and the latter of the Phrynides. This separation seems to be justified on examination of the coxal glands, which differ very widely in these two groups.

*Amblypygi*

The description of the coxal glands of the Phrynides given in the previous article was based upon two species of *Tarantula* from Central and South America. Since then I have had an opportunity of examining several species of *Phryniscus* and of the *Charontini* family from India and the Malay Peninsula.

- |            |  |
|------------|--|
| Phrynini   | <i>Phryniscus ceylonicus</i> , Ceylon.                               |
|            | <i>Phryniscus nigromanus</i> (Gravely), India. <sup>2</sup>          |
| Charontini | <i>Charon grayi</i> (Simon), Manila. <sup>3</sup>                    |
|            | <i>Stygophrynus</i> , caves in Malay Peninsula.                      |
|            | <i>Sarax</i> , caves in Malay Peninsula.                             |
|            | <i>Phrynicho-Sarax singapurae</i> (Gravely), Langkawi Island, Kedah. |
|            | <i>Charinides bengalensis</i> (Gravely), Bengal. <sup>2</sup>        |

The coxal glands of *Phryniscus* are precisely similar to those of *Tarantula* as previously described; i.e., there is a saccule on segment III from which a very extensive coiled labyrinth tube leads backwards to segment VI, where the tube loops forward and runs anteriorly as a long straight tubule to the outlet on segment III, just below the saccule and posterior to appendage III (diagram 1, fig. 1). The labyrinth is peculiar in that the central portion is lined with a basophil epithelium which appears to be secretory, and it was suggested that the secretion might be used for salivary purposes as there is no distinct sali-

<sup>2</sup> Specimens collected and fixed for me by Mr. F. H. Gravely of the Indian Museum, Calcutta.

<sup>3</sup> Specimens given by M. Eugène Simon of Paris. They had been kept in alcohol for twenty years, but were in fairly good condition.



vary gland in *Tarantula* such as is found in the scorpions and the spiders.

The Charontini, like *Tarantula* and *Phrynicus*, have a large coxal gland with saccule and outlet on segment III, the labyrinth of which also possesses special secretory epithelium in its middle portion; but the labyrinth of this large coxal gland does not extend quite so far back as in *Tarantula* and *Phrynicus*.

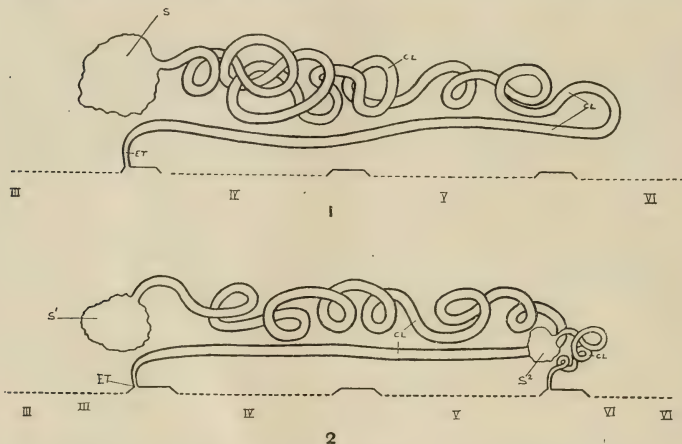


Diagram 1 Coxal glands of the Amblypygi. Fig. 1 Tarantulini and Phrynicini. Saccule on segment III from which the labyrinth extends posteriorly to segment VI, from which point it loops forward and runs as a single tubule to the outlet behind appendage III.

Fig. 2 Charontini. In addition to the large coxal gland on segment III there is a small one, complete in itself, on segment V. S, saccule; CL, labyrinth of coxal gland; ET, exit tubule.

On segment V there is a second, very much smaller gland, complete in itself, with saccule, labyrinth and outlet behind appendage V, but the labyrinth possesses no secretory epithelium (diagram 1, fig. 1).

The presence of this small, apparently disappearing, coxal gland indicates that the Charontini are more primitive than *Phrynicus*, before which they are probably giving way. The

larger genera of the Charontini: *Stygophrynus*, *Charon* and *Sarax*, are found only in caves, while those found in the open, such as *Charinides bengalensis* and *Phrynicho-sarax*, some of which I found on Langkawi Island, are very small, insignificant-looking animals. Langkawi Island lies off the coast of Kedah in the Malay Peninsula, and seems to possess a rather primitive arachnid fauna, as I shall also have occasion to remark in a note on the distribution of the *Uropygi*. *Phrynicho-Sarax* is quite abundant in the jungle on Langkawi Island, but no *Phryniscus* at all were found there.

### *Uropygi*

*Thelyphonides*. The carapace of the older *Thelyphonides* is very hard and difficult to section, but these animals live very well in captivity, and among a number of specimens, kept in small cages and supplied with grasshoppers, one will moult now and then, immediately after which the chitin is thin and soft, and even the mature animal can be readily sectioned. At this stage, also, there is much turgescence of the tissues; the organs are all actively functioning and therefore in a favorable condition for observation.

Species of *Thelyphonides* examined:

|                               |                        |
|-------------------------------|------------------------|
| <i>Thelyphonus linganus</i>   | Malay Peninsula        |
| <i>Thelyphonus sepiaris</i>   | Ceylon (Plains)        |
| <i>Labochirus crassimanus</i> | Ceylon (Hills)         |
| <i>Hypoctonus kraepelini</i>  | Langkawi Island, Kedah |

The coxal glands of all of the above representatives of this family are precisely alike and resemble those of *Tarantula* in that the coils of the labyrinth extend well back into segment VI, and are not concentrated as in the scorpions; but nevertheless, the gland differs greatly from that of *Tarantula*, for there are two saccules—one on segment IV and another on segment V. The saccules, moreover, are elongated and flattened, not roughly spherical like those of *Tarantula* and the other *Amblypygi*. In the case of the *Amblypygi* (and this applies also to the more or less spherical saccule of the scorpions and spiders) the surface

of the saccule is increased by blood capillaries which push in the wall of the saccule and break up its lumen into tortuous channels (figs. 1 and 2). The elongated and flattened saccules of *Thelyphonus* have sufficient surface of themselves and are not invaded by capillaries in this way. The saccule of *Thelyphonus* appears tubular in sections, and the tubules can be distinguished from those of the labyrinth only by the nature of the epithelium lining the walls. The cubical epithelium of the saccule has very delicate outlines and the cytoplasm often contains solid particles—probably urates—which are never found in the coarser striated epithelium of the labyrinth. On injecting carmine the particles are taken up by the cells of the saccules, but never by those of the labyrinth.

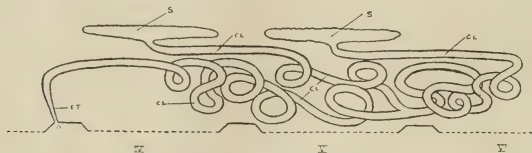


Diagram 2 Coxal gland of the *Thelyphonides*. Two saccules, one on segment IV, and another on segment V, with a labyrinth common to both saccules and an outlet behind appendage III. *S*, saccule; *CL*, labyrinth; *ET*, exit tubule.

From each of the two saccules of *Thelyphonus* a short but wide collecting duct leads into a tubule of the labyrinth directed posteriorly, so that the tubule from the saccule on segment V extends posteriorly into segment VI where it forms coils which ultimately pass forward again. Similarly the tubule from the saccule on segment IV extends backwards into segment V where it joins the main coils. The coils coalesce at some hitherto undetermined point, and the coiled labyrinth tubule passes forward to a point opposite the anterior saccule, where the coiling comes to an end and the labyrinth runs forward as a single straight tubule to its outlet just posterior to appendage III (diagram 2).

The outlet, therefore, is in the same position as that of *Tarantula*, but the saccules, instead of being in segment III only, or in III and V as in the *Charontini*, are in segments IV and V. Moreover, the entire coxal gland is evidently formed by fusion

and modification of three originally distinct glands on segments III, IV and V, whereas the two coxal glands of the Charontini are entirely distinct organs belonging to segments III and V, one of which has been lost in *Tarantula* and *Phrynicus*.

#### Tartarides.

Species examined:

*Schizomus peradeniyensis*, Peradeniya, Ceylon.

*Schizomus vittatus* (Gravely), Peradeniya, Ceylon.

*Schizomus perplexus* n.sp. (Gravely), Plains, Ceylon.

*Schizomus modestus* (Hansen), Kedah, Malay Peninsula.

It is only within the last four or five years that any collector has been able to obtain large numbers of the *Tartarides*. Messrs. Green and Gravely, in the botanical gardens of Peradeniya in Ceylon, used a sieve with which they sifted fallen leaves and débris over a large sheet of stiff paper, the *Tartarides* and small insects falling through the meshes. Adopting this method I was fortunate enough to secure, not only at Peradeniya, but also on the plains of Ceylon and in the Malay Peninsula, nearly 500 specimens of these interesting little animals, of which more than 50 were fixed on the spot and sectioned later. The type species—*S. crassicaudatus*—can not be collected in this way, as it occurs under stones and bricks. A few specimens were secured in Ceylon but they were not sectioned.

Owing to their small size and the minuteness of their organs and individual cells it is not easy to determine the details of the coxal glands, but from a comparison of a large number of specimens one may conclude that the fundamental arrangements of the organ are precisely similar to those obtained in the *Thelyphonides*. There are two flattened tubular saccules, one in segment IV and another in appendage V, from each of which a collecting duet, consisting of five or six minute cells, leads into a labyrinth tubule running posteriorly to segments V and VI respectively. The labyrinth, lined with striated cells, coils very little and is much simpler in construction than that of *Thelyphonus*, but nevertheless it has not been found possible to determine just where coalescence of the two tubules takes place be-



fore the labyrinth runs forward as a single tubule from segment IV to the outlet on segment III.

*Note on the distribution of the Uropygi.* Gravely ('12) suggests that *Hypoctonus* of Burma is hemmed in and is being gradually displaced by the more highly specialized *Thelyphonus sepiaris* of India on the one hand, and *Thelyphonus linganus* of the Malay Peninsula on the other; the latter being closely allied to other species found in the Malay Archipelago, where he supposes that this branch of *Thelyphonus* originated.

The *Hypoctonus kraepelini* found on Langkawi Island is rather small, with red legs; the species having been previously unknown to M. Eugène Simon, to whom I gave some specimens. There is one specimen in the Indian Museum at Calcutta, from the northern (Siamese) region of the Malay Peninsula, found and named by Annandale some years ago, but I do not know of any other, nor is any other species of *Hypoctonus* known to occur outside of Burma.

In Perak and Kedah, British controlled States of the Malay Peninsula, *Thelyphonus linganus* is very common; but I found only one specimen of *Hypoctonus kraepelini*, in the jungle far removed from any settlement, near the Siamese border. On Langkawi Island, only a few miles from the Kedah coast, there are no *Thelyphonus*, but everywhere in the primaeval jungle one can collect numbers of *Hypoctonus kraepelini*, although they do not occur where the jungle has been cleared. The species seems to have been left undisturbed on the island, but on the mainland it has become very scarce, owing to pressure by *Thelyphonus linganus*.

It may also be noted that *Tartarides* (*Schizomus modestus*) are very common in Kedah and Perak on the mainland, but during a month's stay in Langkawi I did not find a single specimen.

#### GROUP II. III. SOLIFUGAE AND PALPIGRADES

##### *Solifugae*

The coxal gland of the Solifugae was fully described in the previous monograph, and a study of a considerable number of additional specimens goes to confirm the results already ob-

tained. Stress was laid on the peculiar nature of the saccule, the lumen of which is filled with a pulpy mass of cells instead of the walls being merely lined with a single layer of delicate epithelium, which is the usual arrangement. This part of the organ had never previously been described, and the opinion expressed, with some reservation, that it is actually the saccule, has since been proved by the injection of carmine into a number of specimens during a visit to Biskra in 1912. The carmine was injected in minute quantities under the thoracic carapace. The animals very quickly recovered from the effects of the inoculation and were killed afterwards at varying intervals of time—from six hours to one month. The carmine is very quickly taken up by the cells of the saccule, and seems to remain there for a long time—even up to a month. No carmine is ever found in any other part of the organ.

The saccule is situated in segment II, the connecting tubule opening into a long narrow sac (labyrinth sac) which extends posteriorly into segment IV, where, after making several coils, it ends blindly. The walls of this part of the gland are lined with secretory epithelium. At a point in proximity to the saccule there is an opening from the labyrinth sac into the true labyrinth, which consists, as in group I, of a long single coiled tubule lined throughout with striated epithelium. The labyrinth extends back as far as segment VI, where it forms several coils and then runs forward again to the exit tubule and outlet on appendage II (diagram 3 fig. 1). In connection with the exit tubule there is a squirting apparatus, apparently intended to force a stream of the secretion towards the prey held in front of the mouth by the chelicerae, but this part of the organ need not be described again in detail. The probable homology of the coxal gland of the Solifugae with the salivary gland of Peripatus was considered and discussed in detail, and I have since seen no reason for changing my opinion.

### *Palpigrades*

*Koenenia mirabilis*. In the spring of 1914 I was able, with the help of Mr. C. Börner of Metz, to collect in the olive groves

of Palmi in Calabria about sixty specimens of *Koenenia mirabilis*, almost all of which were fixed and sectioned for microscopic study. In addition I was able to examine five specimens which had been sectioned and described by Mr. Börner some years previously. The coxal gland had already been observed by Grassi, the original discoverer of *Koenenia*, ('85), and Börner ('04) described it as a single tube running anteriorly from the second

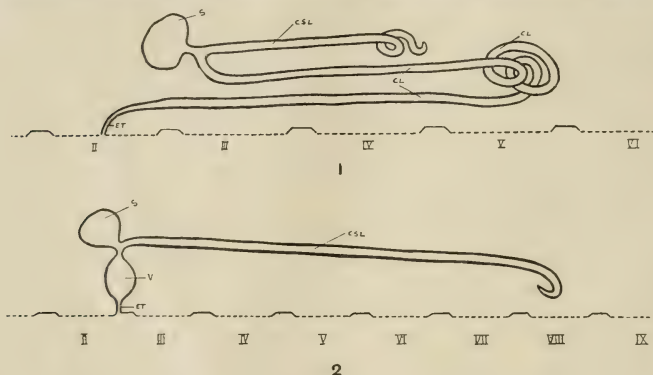


Diagram 3 Coxal gland of group II. Fig. 1 Coxal gland of the Solifugae. Saccule on segment II opening into the long labyrinth sac. From the latter there is an opening into the true labyrinth, running back and then forward again to the outlet on appendage II. In the neighborhood of the saccule the labyrinth sac is pouched and the labyrinth coils a little. For the sake of clearness these pouches and coils are omitted in the diagram.

Fig. 2 Coxal gland of *Koenenia*. Saccule on segment II opening into the long labyrinth sac. The true labyrinth is represented only by the vesicle, the outlet from which is on appendage II. *S*, saccule; *CSL*, labyrinth sac; *CL*, labyrinth; *ET*, exit tubule; *V*, vesicle.

abdominal (genital) segment to an outlet just posterior to appendage II. This description is correct so far as it goes, but Börner overlooked the saccule and did not appreciate the significance of the tube being single and not double. He was not acquainted with the anatomy of the Solifugae and his efforts were chiefly directed towards grouping *Koenenia* with the Pedipalps.



As *Koenenia* is exceedingly minute, its organs and the cells composing them are all on a correspondingly minute scale, so that it is not by any means easy to make out the different parts of the gland, and it is only by studying a large number of specimens that one can come to satisfactory conclusions. There is a small circular saccule in segment II consisting of eight or ten fairly well defined cells enclosing a lumen, from which a short collecting duct of five or six cells leads into a long narrow sac which extends posteriorly into the abdomen to segment VIII (genital), where it forms a short loop and ends blindly. As already observed by Börner, the cells of this posterior loop differ from those of the straight part of the sac, and he quite rightly remarks that none of the cells, either in the loop or in the straight part of the sac, have a striated base; i.e., they are secreting, not excreting cells such as one finds in the true labyrinth of the arachnids. Just below the point at which the collecting tubule or duct changes to the long sac, there is an opening leading into a dilated vesicle, and from the vesicle there is a short exit tubule leading to the outlet just posterior to appendage II (diagram 3, fig. 2).

It seems obvious that this organ must be homologous with the coxal gland of the Solifugae rather than with that of the scorpions and spiders. The saccule and outlet are on the same segment (II), and we also find the same long sac with a coil at its posterior extremity extending far back and lined with secreting cells. In *Koenenia*, however, the true labyrinth has practically disappeared as such, being represented merely by the vesicle, which probably only acts as a reservoir for the products of secretion, having no excretory functions, since the walls of the vesicle do not appear to be lined with striated epithelium.

It may be further remarked that if this long sac represented the true labyrinth, not only should it be lined with striated cells but it would necessarily have to loop forward on itself and run forward again to the outlet, thus forming a double tubule on cross section at any point; but it is a single tube throughout and has been correctly so described by both Grassi and Börner.

The coxal glands of the Arachnids have always been described as being located in such and such a segment, but it is probable that fundamentally each coxal gland represents two segments instead of one. There seems little doubt that in the saccule we have the last traces of the coelom, which would belong to the segment anterior to that of the main part of the coxal gland. One may reflect that in the worms the coelom of one segment is connected with the segment next posteriorly to it by means of a funnel represented by the collecting tubule of the arachnids. The funnel leads through the partition into a coiled nephridium situated in the next segment; the coiled tubule of the nephridium

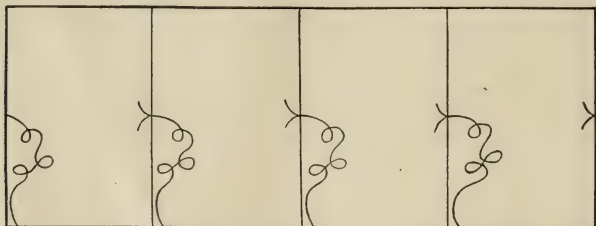


Diagram 4 Four segments of a worm, showing the nephridia.

having an outlet near the anterior partition of the segment (diagram 4). When, therefore, dealing with the arachnids, we say, as for instance with *Phrynicus*, that the saccule lies in segment III and the gland has its outlet on segment III just behind appendage III, it should be borne in mind that the saccule and collecting duct represent the coelom and funnel of segment III, while the labyrinth and outlet belong in reality to segment IV; the labyrinth having encroached upon and suppressed the coxal glands immediately posterior to itself—a process of encroachment which is still in progress in the Charontini, the small posterior gland of which is composed of the coelom and funnel of segment V and the nephridium of segment VI.

## PART II

## THE GANGLIA OF THE ARACHNID

If the cephalothorax of an arachnid such as the scorpion or Thelyphonus be sectioned sagittally through the median line, the ganglia composing the large suboesophageal ganglion can be clearly distinguished, the suboesophageal ganglion being mapped out into a number of neuromeres, separated from each other by a small artery whose course can be very readily determined. Each of these neuromeres represents one of the individual ganglia of which the whole is composed. In the same way also it can sometimes be recognized that the abdominal ganglia are composed of more than one individual ganglion. By enumerating the ganglia in this way it is determined that there are eighteen ganglia in each of the four orders which go to form group I of the arachnids: scorpions, spiders, Uropygi and Amblypygi. One of these eighteen ganglia (the cheliceral) has moved up and fused with the supraoesophageal ganglion; the other seventeen being found in the suboesophageal ganglion and in the abdomen.

## GROUP I. IV. SCORPIONS

The nervous system of the scorpions is less concentrated than that of the other arachnids. The suboesophageal ganglion consists of nine neuromeres, the ninth neuromere supplying the abdomen as far as the first lung (fig. 5). In the abdomen itself there are three single ganglia supplying lungs 2, 3 and 4. In the post-abdomen are four ganglia, of which the last is double, having two neuromeres. The entire ventral nerve chain, therefore, consists of seventeen ganglia which, with the cheliceral ganglion, fused with the brain, would make eighteen.



## V. PEDIPALPS

*Amblypygi*

The ganglia are all concentrated in the cephalothorax, the suboesophageal ganglion containing seventeen neuromeres. The photograph is taken from the only section I have which shows the whole of the neuromeres in one field, but in a large number of specimens it has been found easy to detect the whole seventeen by making a composite drawing from several sections in series. The Tarantulini Phrynini and Charontini are precisely similar as regards this arrangement of the ganglia (figs. 3 and 4).

*Uropygi*

Thelyphonides. Both in young and adult specimens the suboesophageal ganglion contains only twelve neuromeres, but at the posterior end of the abdomen there is a large additional ganglion with five neuromeres, representing the last five abdominal segments.

The eggs of *Thelyphonus* (*linganus*, the species studied) are carried in a packet attached to the underside of the abdomen of the mother. Just after hatching out, the larvae, white and inert, are carried wrapped around the body of the mother to which they are firmly attached by a gummy substance. At this stage the suboesophageal ganglion consists of nine neuromeres; the other eight ganglia being paired and strung out separately along the abdomen. In a few days, however, and before the next moult, the ganglia have become concentrated; the three anterior pairs becoming fused in the median line and passing forward to join the suboesophageal ganglion, while the five posterior pairs also fuse in the median line and with each other to form the abdominal ganglion as in the more mature animals. Strubell ('92), for *Thelyphonus Caudatus*, described six pairs of ganglia in the cephalothorax and ten pairs in the abdomen—his specimens probably representing a slightly earlier stage than I had an opportunity of observing; but there are certainly seventeen ganglia altogether, and not sixteen as he described.

Tartarides. The arrangement of the ganglia in the Tartarides at first sight appears to differ fundamentally from that in the Thelyphonides, since there is, in addition to the suboesophageal, a large ganglion situated in the anterior part of the abdomen in the neighborhood of the genital segment instead of at the posterior end of the abdomen. Microscopical examination, however, shows that there are nine neuromeres in the suboesophageal ganglion and eight in the abdominal ganglion, making seventeen in all—the same number as in the Thelyphonides—and it may be presumed that the Thelyphonides and Tartarides became differentiated from a common ancestor at a stage when there were still eight separate paired ganglia in the abdomen; a stage now represented in the larval Thelyphonides. On further specialization the ganglia in the Tartarides became concentrated into a single anterior abdominal ganglion, while in the Thelyphonides two groups of three and five ganglia respectively were formed, the first group passing forward to join the suboesophageal ganglion and the latter remaining in the posterior part of the abdomen.

It may be recalled that, apart from their external affinities, which have led to placing these two groups in the same order, the close relationship of the Thelyphonides and Tartarides is clearly established by the similarity of the coxal glands—a fact which makes it easier to accept this explanation of the apparently fundamental difference in the arrangement of the ganglia.

## VI. SPIDERS

In all spiders there appear to be twelve neuromeres in the suboesophageal ganglion, although with the *Araneae verae* it is not often possible to make them out except in larval and quite young specimens, but the Theraphosid spiders show the neuromeres more clearly, always to the number of twelve.

In young and adult spiders there are no abdominal ganglia, nor have I ever found any trace of them in larval specimens of the *Araneae verae*; but in 1913 I was fortunate enough to find in Sumatra a cocoon of a large *Chilobrachys* (Theraphosid) containing nearly a hundred larvae just after hatching, and

these I was able to keep alive for two months, fixing four or five for sectioning every few days. In the larval stage the spiders were very inert, with distended abdomen containing a large amount of yolk. They were not pigmented and remained quiescent in the cocoon. At the end of a month they all moulted simultaneously and left the cocoon, passing from the larval to the immature stage. They were now pigmented, long-legged, with small abdomen, and very active, remaining so until the last of them had been killed for sectioning a month later. These points are merely mentioned to show that the spiders were in a normal condition, at any rate in the early stages.

In the youngest specimens five abdominal ganglia were clearly distinguishable, but the ganglia rapidly disappeared and in about a week there were no traces left. I do not think that any observation of these transient abdominal ganglia in spiders has ever been recorded.

In specimens killed at once and two days later, the suboesophageal ganglion contains twelve neuromeres very clearly mapped out. From its posterior end two parallel nerve cords pass through the pedicle, beyond which they separate, running laterally and ventrally in the abdomen. On either side of the abdomen are three very clearly defined, large ganglia, connected together by the nerve cord. The nerve cord can be traced a little beyond the third ganglion, where it is lost, but more posteriorly there are two very much smaller ganglia lying in the neighborhood of the intestinal tract, which has so far only partially been formed. The rest of the abdomen is filled with yolk (diagram 5).

In specimens fixed three days later (five days after finding the cocoon) the nerve cord was no longer traceable into the abdomen; the two posterior ganglia had disappeared and the three anterior were greatly diminished in size.

On the seventh day there were some traces of one or two ganglia, but it was difficult certainly to identify them, and this was the last trace of them that could be found. If the series had begun at this stage the ganglia would certainly have been overlooked or not recognized as such.

With the twelve neuromeres of the suboesophageal ganglion these five paired evanescent ganglia in the abdomen make up the usual number of seventeen for group I of the arachnids, which may here be tabulated.

|                    | BRAIN      | SUBOESO-<br>PHAGEAL | ABDOMINAL GANGLIA          |           | TOTAL |
|--------------------|------------|---------------------|----------------------------|-----------|-------|
|                    | Chelicerae | Cephalo-<br>thorax  | Anterior                   | Posterior |       |
| Scorpions.....     | 1          | 9                   | 1 1 1                      | 1 1 1 2   | 18    |
| Amblypygi.....     | 1          | 17                  | 0                          | 0         | 18    |
| Thelyphonides..... | 1          | 12                  | 0                          | 5         | 18    |
| Tartarides.....    | 1          | 9                   | 8                          | 0         | 18    |
| Spiders.....       | 1          | 12                  | (1 1 1 1 1)<br>(Transient) |           | 18    |
|                    | 6 segments |                     | 7 segments 5 segments      |           |       |

#### GROUP II. VII. SOLIFUGAE AND PALPIGRADES

The Solifugae being tracheate animals, there are no blood channels to divide the neuromeres, although in the cephalothorax their place seems to be taken to some extent by tracheae. By means of the tracheae and the nerves proceeding from the suboesophageal ganglion, one can determine that the suboesophageal ganglion is composed of five neuromeres supplying appendages II to VI. The nerve supply to appendage VI, on which the racquet organs are situated, is very abundant, and is supplemented by a number of secondary ganglia at the roots of the racquets themselves.

With the neuromeres supplying appendage VI, however, the suboesophageal ganglion appears to come to an end, and from this point there is a large nerve cord leading into the anterior part of the abdomen where it swells into a large single ganglion in the region of the genital segment. The nerve cord emerges from this ganglion posteriorly, and can be traced a short distance after which it becomes distributed into small filaments and gradually disappears. There is no trace of any ganglion in the abdomen other than the one mentioned, nor does this ganglion show any evidence of being divided into neuromeres. It is simply one solid ganglionic mass (diagram 6, fig. 1).



*Palpigrades*

Koenenia, the coxal gland of which has been shown to be closely related to that of the Solifugae, also shows a very similar arrangement of the ganglia. In the suboesophageal ganglion there are five swellings on either side, one for each corresponding appendage, and from the posterior end of the ganglion a nerve cord passes to the abdomen, in the anterior part of which there

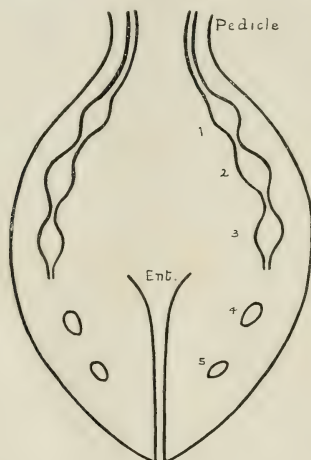


Diagram 5 Evansecent abdominal ganglia of Chilobrachys. Five ganglia on either side of the abdomen, the first three of which are connected by a nerve cord running through the pedicle from the suboesophageal ganglion. *Ent.*, intestine.

are three distinct ganglionic swellings, beyond which the nerve cord can not be traced. These three ganglionic swellings in the genital region appear to correspond to the abdominal ganglion of the Solifugae, in which they have become concentrated into a single mass (diagram 6, fig. 2).

*Note on the segmentation of the arachnids.* To return to group I, we find in each case that there are eighteen ganglia, and the question naturally arises—to what extent do these ganglia represent segments?

The supraoesophageal ganglion, with its sense organs, need not be considered as having any segmental value except in so far as the ganglion for the chelicerae is concerned—a ganglion which in the earliest stages is obviously suboesophageal and on later development moves over and becomes fused with the brain.

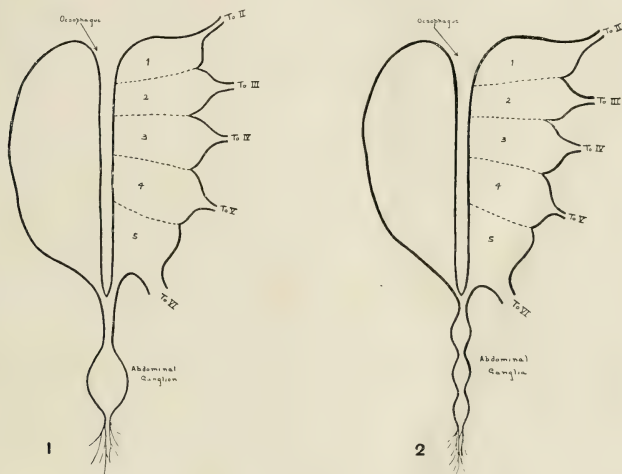


Diagram 6 The ganglia of group II. Fig. 1 Frontal view of the ganglia of the Solifugae. The five neuromeres of which the suboesophageal ganglion is composed are indicated, and below is the large single abdominal ganglion, connected by a nerve cord with the suboesophageal.

Fig. 2 Frontal view of the ganglia of *Koenenia*. Resembling that of the Solifugae, but showing three abdominal ganglia instead of a single one.

The five anterior ganglia of the suboesophageal mass supply appendages II to VI, which, with the chelicerae, represent the six segments of which the cephalothorax is composed. This number of segments of the cephalothorax obtains in all orders of this group and leaves twelve abdominal segments to be accounted for, each with its attendant ganglion.

*Pedipalps*

In the Pedipalps, both Uropygi and Amblypygi, the relations are clear enough. There is a small first abdominal segment (VII), after which comes the second abdominal, or genital segment (VIII); posterior to the genital segment are ten more segments, making twelve abdominal segments in all; the number of segments therefore corresponding to the number of ganglia—six for the cephalothorax and twelve for the abdomen.

*Spiders*

In the ordinary spiders all trace of segmentation of the abdomen is lost, but the pedicle is taken to represent a first abdominal segment (VII) after which comes the genital organ representing segment VIII. Liphistius has nine tergal plates on the abdomen, each of which is presumed to represent a segment, so there are still three segments wanting. McBride ('14), quoting Kishinouye, says that in the earlier stages of development of Theridion ten segments are marked out in the abdomen, of which the tenth is not always distinct, nor does it always develop a coelomic cavity. There is no proof, therefore, of correspondence between the number of ganglia and segments in the spiders, although, since the rudiment of the tenth abdominal segment is not always laid down, it is possible that an eleventh and a twelfth segment may have originally existed but have now entirely disappeared.

*Scorpions*

There is not complete correspondence between the ganglia and the segments in the scorpions, although the total number of eighteen is the same. The cephalothorax is composed of six segments as usual, and the abdomen of twelve, but the first abdominal segment (VII) has disappeared, leaving the genital as the first abdominal segment, posterior to which are the segments bearing the pectens, the four lungs and the last abdominal segment—making seven in all—while beyond this comes the post-abdomen with five segments forming the tail. There are,

therefore, eleven post-genital segments instead of ten as in the Pedipalps.

The first abdominal segment has disappeared, although Brauer ('95) found a transient rudiment corresponding to it in the embryo scorpion.

On examination of the suboesophageal ganglion it is found that the neuromeres 1 to 5 supply appendages II to VI; the seventh neuromere supplies the genital organ, the eighth neuromere the pectens and the ninth the first lung—leaving the sixth neuromere, which should supply the missing segment VII, to be accounted for. This sixth neuromere is clearly mapped out and is quite normal in size, but I have not been able to trace nerves preceding from it. It seems probable, however, that it may be used as supplementary to the pectens, for the nerve supply of which there is a secondary paired nervous mass lying ventrally to the suboesophageal ganglion as shown in diagram 7.

Just ventrally to the eighth neuromere, on either side of the median line, there is a large mass of nerve fibers from which a cord runs posteriorly and joins the nerve from the eighth neuromere, and another cord runs anteriorly to a point opposite the sixth neuromere where there is another large swelling. I have not been able to detect any direct connection between the sixth neuromere and the nerve mass just ventral to it, but it seems probable that there must actually be some such connection and that the sixth neuromere, after the disappearance of segment VII, became utilised as a secondary source of supply for the pectens.

In the Buthidae, but not in the Scorpionidae, Chactidae, or Vejovidae, there is a further extension of this ventral nerve mass anteriorly to about opposite the second neuromere, where there is another small swelling but no direct connection with the suboesophageal ganglion itself. (This extension is shown in diagram 7.)

Whatever may be the true function of the sixth neuromere, it is clear that it is interpolated between the fifth and seventh without having any corresponding segment to supply. Segment VII has disappeared, but the ganglion which originally supplied it remains.



Turning now to the abdomen we find twelve segments, of which the first is the genital, but there remain only eleven ganglia to supply them since we have already disposed of seven ganglia (segments I to VII) (VII being transitory). The ganglia of the genital organ (VIII), pectens (IX) and first lung (X) are fused with the suboesophageal ganglion. Next comes a

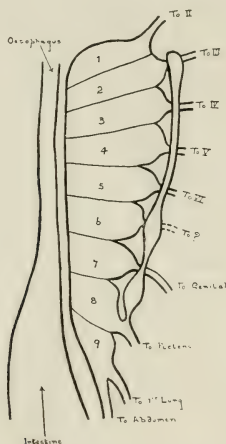


Diagram 7 Sagittal view of the suboesophageal ganglion of the scorpion, showing nine neuromeres, the destination of the nerves proceeding from them; and the ventral nerve mass secondarily supplying the pectens. It is understood that all these structures can not be seen in one section. The neuromeres can only be distinguished in sections through the median line, whereas the nerves running to the appendages and the secondary nerve mass for the pectens are paired structures which appear in sections on either side of the median line.

series of three single ganglia for lungs 2, 3 and 4 (XI, XII, XIII), posterior to which is the last abdominal segment (XIV). In the post-abdomen there are five segments and four ganglia, of which the last, situated in the fourth segment, is double (having two neuromeres) and clearly supplies segments 4 and 5. The last segment in the abdomen, therefore, appears to be without a ganglion. The first ganglion of the post-abdomen is cer-

tainly single,<sup>4</sup> but it lies in the anterior part of this segment and extends anteriorly through the constriction into the last segment of the abdomen in the egg just before hatching. I have not had an opportunity of examining earlier stages. The suggestion may be put forward that the last abdominal and first post-abdominal segments represent in reality a single segment which has become secondarily divided into two in order to give to the base of the tail greater strength than could be obtained by utilizing the division between two primary segments. This suggestion seems to receive some support from Brauer's figures of the development of the scorpion, a part of one of which is reproduced in diagram 8. Brauer, in his text, treats segments XIV and XV (first post-abdominal) as if they are two distinct segments, but it appears from his figures as if segment XIV folds over on itself to form the base of the tail, without at first any segmentation at the point of the fold.

That such a division is possible is indicated by the secondary division between the fifth post-abdominal segment and the poison gland, which corresponds to the telson of *Limulus* and the Eurypterides. The telson is not considered as representing an original segment, since it always lies behind the anus. There is no ganglion for the poison gland in the scorpion. Similarly the Uropygi and *Koenenia* have a tail jointed to the last abdominal segment, but the tail is not recognized as a segment, nor has it any corresponding ganglion.

The scorpion-like Eurypterides of the Silurian have ten post-genital segments—one less than the scorpion; the pre-genital segment (VII) being recognizable on the dorsal surface, but on the ventral surface it is fused with the genital plate. On the other hand, the Silurian scorpion, *Palaeophonus*, appears to have the same number of segments as the modern scorpions.

The general conclusion is that in group I of the arachnids the number of ganglia corresponds to the number of segments—eighteen of each—obvious in the Pedipalps; not so clear, but probable, in the scorpions; in the spiders possible but not proven.

<sup>4</sup> I have looked in vain through scores of specimens for some indication of division of this ganglion into two neuromeres.

In regard to group II, since the large abdominal ganglion of the Solifugae is a single, solid mass, without any evidence of neuromeres, nothing definite can be said about it. It may represent a fusion of all the original abdominal ganglia, or it may be composed of one or more of the original ganglia which have suppressed and taken over the functions of others. The same may be said of the three abdominal ganglia of the Palpi-

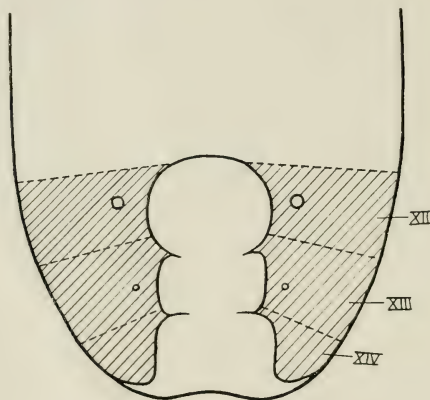


Diagram 8 From Brauer. Development of the tail of the scorpion. Between segments XIV (last abdominal), and XV (first post abdominal), there is no evidence of segmentation. Segment XIV seems to double over on itself to form the base of the tail. In the tail itself only three out of the five segments have been cut out at this stage.

grades. In neither of these two orders can any relation between the abdominal segments and ganglia be determined. In this respect they differ again from group I; and a still further difference between the two groups can be pointed out; in each of these two orders of group II there are nine post-genital segments instead of ten, as is probable in all the orders composing group 1.

*Tabulation of differences between group I and group II*

|                                | GROUP II   | GROUP I             |
|--------------------------------|------------|---------------------|
| Coxal gland saccule.....       | Segment II | Segments III, IV, V |
| Coxal gland outlet.....        | Segment II | Segments III, V     |
| Coxal gland labyrinth sac..... | Present    | Absent              |
| Ganglia.....                   | 7 or 9     | Always 18           |
| Ganglia neuromeres.....        | Doubtful   | Clearly marked      |
| Segments, post genital.....    | 9          | 10                  |

My best thanks are due to Professor Pruvot for permission to work in his laboratory of comparative anatomy at the Sorbonne, and also to M. Eugène Simon and Mr. F. H. Gravely for specimens and suggestions. The diagrams were made for me by Miss G. E. Carver.

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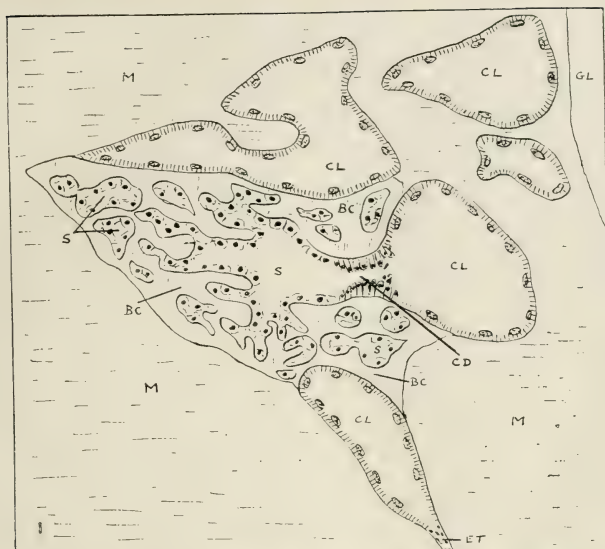


## PLATE 1

### EXPLANATION OF FIGURES

1 *Tarantula palmata*. Saccule of the coxal gland.  $\times 175$ . The drawing is outlined from a photograph, the cells being drawn in semi-diagrammatically, to show more clearly than is possible in photograph the differences between the cells of the saccule, the collecting duct and the labyrinth. The lumen of the saccule is broken up into channels by blood capillaries or rather lacunae) which push in the walls, and thus increase the surface of the saccule. In the diagrams this feature of the saccule is not shown. *S*, saccule; *CD*, connecting duct; *CL*, labyrinth; *ET*, commencement of exit tubule; *M*, muscle and connective tissue indicated by horizontal broken lines; *BC*, blood capillaries indicated only by vertical broken lines; *GL*, suboesophageal ganglion.

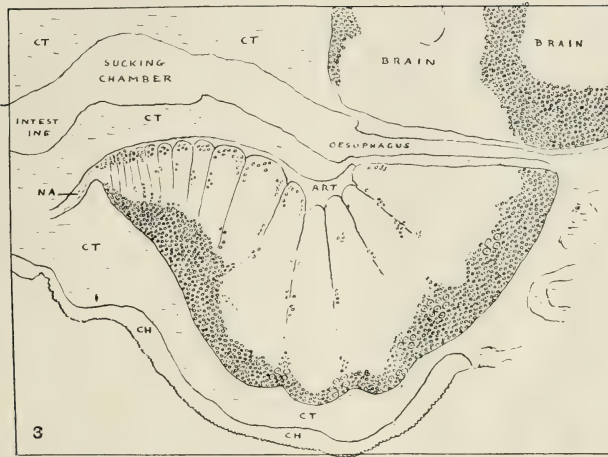
2 *Vejovis flavescens*. Photograph of coxal gland of scorpion.  $\times 100$ . With the help of figure 1 the constituent parts can be made out. In the center is the saccule; its lumen, broken up by inhangng capillaries, opening on the right into the labyrinth, whose tubules in this case completely surround the saccule except at one point (*CA*) where the blood supply enters. At *ET* is the commencement of the exit tubule, but the tubule itself is not shown. The coxal gland rests posteriorly (right) upon the diaphragm *D* which separates the cephalothorax from the abdomen. *C*, caeca of the intestine; *N*, nerve supplying appendage VI; *M*, muscle; *CA*, coxal artery; *ET*, exit tubule; *D*, diaphragm.



## PLATE 2

1 *Tarantula palmata*. Neuromeres of the suboesophageal ganglion.  $\times 60$   
Sagittal section through median line of cephalothorax outlined from the photograph showing 17 neuromeres. Anterior to the suboesophageal ganglion the tissues are broken up on account of extraction of the chelicerae, which are too hard to section. *ART*, artery, the branches from which run between the neuromeres; *CH*, chitin of external surface; *CT*, muscle and connective tissue indicated by broken horizontal lines; *NA*, nerve to abdomen.

Fig. 2. Photograph from which the drawing was outlined.

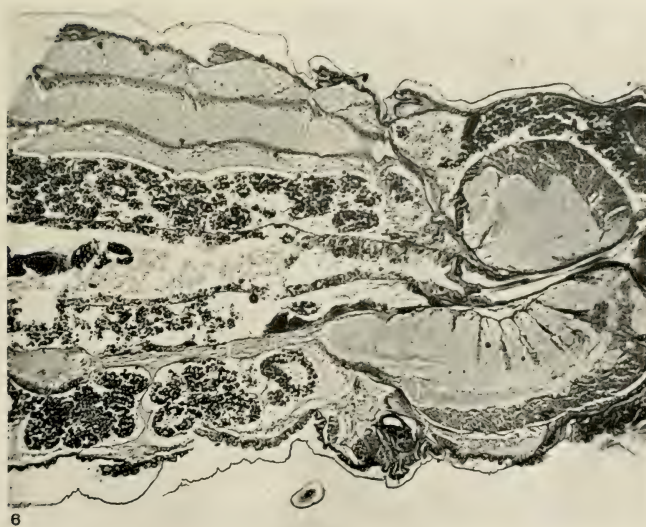
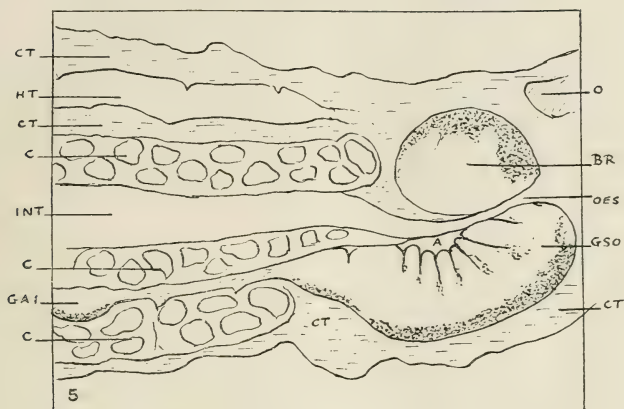




### PLATE 3

1 Immature scorpion. *Buthus occitanus*. Neuromeres of the suboesophageal ganglion.  $\times 40$ . Sagittal section through median line of cephalothorax outlined from the photograph showing 9 neuromeres, of which the 6th is well defined and normal in size. *A*, artery from which branches run between neuromeres; *BR*, brain; *C*, caeca of the intestine; *CT*, connective tissue and muscles; *GSO*, suboesophageal ganglion; *GA*<sub>1</sub>, 1st abdominal ganglion; *HT*, heart; *INT*, intestine; *OES*, oesophagus; *O*, median eye.

2 Photograph from which the above drawing was outlined.





# THE OLFACTORY ORGANS OF LEPIDOPTERA

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## TEN FIGURES

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## INTRODUCTION AND METHODS

In the investigation herein recorded a careful study of the morphology of the olfactory pores of Lepidoptera has been made in order to determine whether these organs are better adapted anatomically than the antennal organs to receive olfactory stimuli.

The investigators who have performed experiments on butterflies and moths with mutilated antennae have concluded that these appendages bear the olfactory organs, regardless of whether or not the antennal organs are anatomically fitted to receive olfactory stimuli. Since these investigators failed to study sufficiently the behavior of the insects investigated, it is possible that the responses observed misled them in determining the seat of the olfactory organs (see the author's paper, '14 c).



In 1857 Hicks discovered pore like organs on the wings of Lepidoptera and called them vesicles. He says: "In moths they are very apparent, being greatest in the noctuae [Noctuidae] and Bombycidae. There are about 100 vesicles on the upper surface of the posterior wing, and half that number beneath, besides some few on the nervures [veins]. In the butterfly they are smaller, but arranged in more definite groups, about three in number." In 1860 Hicks discovered them on the legs of Lepidoptera, and the present writer ('14 a and b, '15 and '16) has made a comprehensive study of these organs in Hymenoptera and Coleoptera, and since he proved experimentally that they receive olfactory stimuli, they were called olfactory pores. On account of other investigations, the writer has not had time to determine the physiology of these organs in Lepidoptera and for this reason the present paper deals with only their morphology.

To obtain material for the study of the disposition of the olfactory pores, dried museum specimens were mostly used. In regard to preparing the specimens with caustic potash and to bleaching them with chlorine gas, the reader is referred to the writer's work on Hymenoptera ('14 b, p. 295).

To obtain material for the study of the internal anatomy of the organs herein discussed, moths emerged a short time from the cocoons and a butterfly were used. The material was fixed in Carnoy's fluid (equal parts of absolute alcohol, chloroform, glacial acetic acid, with corrosive sublimate to excess) and was embedded in celloidin and paraffin. The sections were cut five and ten microns in thickness and were stained with Ehrlich's hematoxylin and eosin.

All the drawings were made by the writer and all are original except figure 10, which represents the antennal organs of a moth and was copied from Schenk ('02). The drawings were made at the base of the microscope with the aid of a camera lucida.

The museum specimens were secured through the courtesies of Dr. L. O. Howard and Mr. August Busck, and the writer is also grateful to Mr. Carl Heinrich for verifying the identification of all the Lepidoptera used.

## THE OLFACTORY PORES

Before making a study of the anatomy of the organs, called the olfactory pores by the writer ('14 a), the distribution and number of them were first investigated.

*Disposition*

In making a comparative study of the disposition of the olfactory pores in Lepidoptera, 40 species, belonging to 36 genera and representing 19 families, were used. In most cases only one specimen of each species was employed, and whenever a portion of an appendage or an entire appendage was missing or was badly mutilated in being prepared for study, the supposed number of pores on this portion or entire appendage was regarded the same as the number found on the corresponding portion or entire appendage on the opposite side of the body. Since the pores on only one specimen for each species were counted, the total number of pores recorded can not be a fair average. Besides this error, there is also another small probable error for each species, because a few of the pores were probably overlooked, and often, as on the tibiae, it was impossible to distinguish the olfactory pores from hair sockets. Only the legs, wings and mouth parts were examined, although in two or three instances the chitinous parts of the reproductive organs were also examined, but no olfactory pores were seen on them. The mouth parts of more than one-half the specimens were either missing or were so mutilated that the pores on them could not be counted; nevertheless, regardless of all the errors, the total numbers of pores recorded are probably not far from being accurate, but they are perhaps always slightly less than the numbers actually present. The sex of the species, except in four cases, was not determined.

a. *Bombyx mori* ♀. Since the silkworm moth, *Bombyx mori*, is conveniently studied and, as its olfactory pores are typical for most of the moths examined, the disposition of them will be described in detail, and then the variations found in the other species will be given.

The wings have dorsal and ventral surfaces, and the legs may be divided for description into two surfaces. The inner surface faces the body of the insect and the outer surface is directed away from it. On the specimen examined, four groups of pores, besides many scattered pores, were found on each front wing; three groups, besides several scattered pores, on each hind wing; and four groups, besides two isolated pores on each leg.

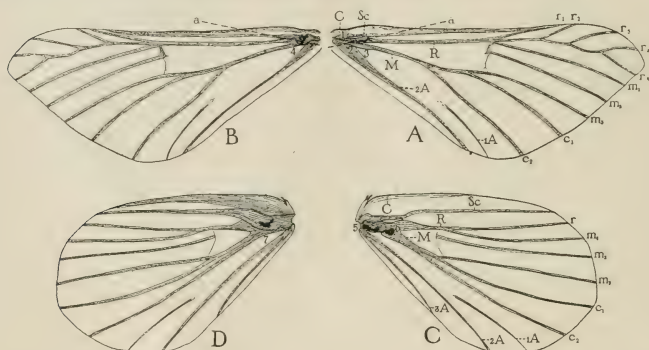


Fig. 1 Wings of silkworm moth, *Bombyx mori* ♀, showing location of groups of olfactory pores as indicated by the numbers 1 to 7, and disposition of scattered pores on the veins as represented by dots,  $\times 3$ . A and B, dorsal and ventral surfaces of right front wing respectively; and C and D, dorsal and ventral surfaces of right hind wing respectively. 1 to 3 A, first to third anal veins; C, costa; c1 to 2, first and second cubital veins; M, media; m1 to 3, first to third medial veins; R, radius; r1 to 5, first to fifth radial veins; Sc, subcosta.

For descriptive purposes in locating the olfactory pores on the wings, the veins at the bases of the wings may be called costa (fig. 1 A-D, C), subcosta (Sc), radius (R), media (M), and anal vein (1 to 3 A); the names, given by Comstock (manual, '01) to the distal ends of these veins, are also appended, so that no confusion may arise. The groups and scattered pores are located as follows: Groups nos. 1 to 7 lie on the bases of the wings, nos. 1 to 4 being on the front wing and nos. 5 to 7 on the hind wing; nos. 1, 2, 3, 5 and 6 are found on the dorsal surfaces while nos. 4 and 7 lie on the ventral surfaces. Nos. 1 to 3 (fig.

1 A) seem to lie on the union of the radius and media, but cross sections (figs. 6 A and 9 C) show that nos. 1 and 2 lie on the media while no. 3 lies on the radius. No. 4 (fig. 1 B) lies on the broad portion of the subcosta which unites with the radius and media. No. 5 lies on the radius and no. 6 (fig. 1 C) on the media, but perhaps no. 6 lies more correctly on the union of the radius and media (fig. 6 B). No. 7 (fig. 1 D) is located on the broad portion of the subcosta.

The number of pores in groups nos. 1 to 7 on the right wings are as follows: No. 1—11, no. 2—52, no. 3—56, no. 4—67, no. 5—128, no. 6—44, and no. 7—12; on the left wings: no. 1—10, no. 2—50, no. 3—52, no. 4—74, no. 5—143, no. 6—51, and no. 7—11. On the dorsal surface of the right front wing, the scattered pores are located as follows: 2 at the distal end of the costa (fig. 1 A), 10 extending the full length of the subcosta, 2 near the base and 5 at the distal ends of the radial branches, 6 at the distal ends of the medial branches, and 2 at the distal end of the anal vein; on the ventral surface of the same wing: 8 extending one-half the length of the costa (fig. 1 B), 5 near the middle of the radius and 8 at the distal ends of its branches, 6 at the distal ends of the medial branches, and 2 at the distal end of the radial vein. On the dorsal surface of the right hind wing, the scattered pores are as follows: 3 near the base of the subcosta (fig. 1 C), 1 at the distal end of a radial branch, 6 near the base of the media, and 6 on the medial branches; on the ventral surface of the same wing: 1 near the base of each the costa and subcosta (fig. 1 D), 1 at the distal end of a radial branch, 4 on a medial branch, and 2 at the distal end of the second branch of the anal vein. The disposition of the scattered pores on the left wings is very similar to that just given for the right wings.

Groups nos. 8 and 9 of the olfactory pores lie on the outer surface of the trochanter (fig. 2 E) of each leg near the anterior margin, while no. 10 is located on the inner surface of the same segment (fig. 2 F). No. 11 lies at the proximal end of the femur (fig. 2 F) of each leg on the inner surface near the posterior margin, and the two isolated pores (fig. 2 E, a) lie at the same position on the outer surface of each leg.



The number of pores in each group on the right leg is as follows: Front leg, no. 8—10, no. 9—8, no. 10—9, no. 11—9; middle leg, no. 8—11, no. 9—6, no. 10—6, no. 11—8, hind leg, no. 8—11, no. 9—6, no. 10—8, and no. 11—8. The variation in the number of pores on the left legs is very similar to that just given.

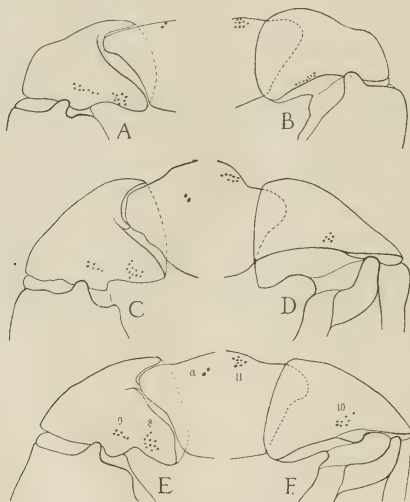


Fig. 2 Portions of legs of *Bombyx mori* ♀, showing location of groups nos. 8 to 10 of olfactory pores on the trochanters, no. 11 and *a* on the femurs,  $\times 192$ . A and B, outer and inner surfaces of right front leg respectively; C and D, same of right middle leg; and E and F, same of right hind leg.

All six legs of the specimen of *Bombyx mori* ♀ examined bear 218 olfactory pores; the front wings carry 423 pores, and the hind wings carry 420 pores; no pores were observed on the mouth parts; all of these combined make 1061 olfactory pores.

*b. Other species.* The greatest variation found in the olfactory pores of the other species examined is in regard to the total numbers of the pores. On the average, butterflies have only two-thirds as many pores as have moths; this difference is due solely

to the smaller number of pores on the wings of butterflies, and chiefly to the smaller number on their hind wings. The preceding conclusion was derived from the following data: The total number of pores on the legs of moths vary from 71 to 240, with 140 as an average; on the legs of butterflies from 73 to 196, with 141 as an average; on the front wings of moths from 52 to 662, with 399 as an average; on the front wings of butterflies from 206 to 404, with 303 as an average; on the hind wings of moths from 45 to 663, with 382 as an average; on the hind wings of butterflies from 130 to 228, with 197 as an average; on the mouth parts of moths from 0 to 59, and on the same appendages of butterflies from 0 to 34. The total number of pores on moths vary from 222 to 1422, with 930 as an average; and on butterflies from 514 to 784, with 645 as an average.

As a general rule, all the other insects hitherto examined for olfactory pores by the writer showed that the larger the species the greater was its total number of pores. Relative to Lepidoptera this ruling is not true, because the larger species have about the same number of pores as have the smaller species (table 1, p. 43).

The other variations, most of which are small, pertain chiefly to the distribution of the olfactory pores. For sake of brevity, instead of using the long scientific names of the Lepidoptera examined, the species will be numbered from 1 to 43, and those interested in associating the names of the species with the variations described may do so by referring to the names and numbers of the species in the table on page 43.

No wing was found devoid of olfactory pores, although they are reduced in number on the rudimentary wings of the females of *Hemerocampa* and *Alsophila* (nos. 10 and 16). These wings will be discussed under sexual variations on page 41. The disposition of the pores on the wings of the other specimens is more or less similar to that already described for *Bombyx mori*. The number of groups of pores on a wing depends on how closely the pores lie to one another. At a given place on one wing the pores may be scattered and therefore do not constitute a group, while on another wing at the same place, the

pores are well grouped. This fact chiefly explains the variation in the number of groups of pores. Two specimens examined (Nos. 16, 18) have only two groups on each front wing and one group on each hind wing; three specimens (nos. 15, 17, 26) have three groups on each front wing and two on each hind wing; 11 specimens (nos. 1, 3, 5, 6, 8, 9, 11, 12, 19, 20, 43) have three groups on each front wing and three on each hind wing; one specimen (no. 25) has four groups on one front wing and two on the other, two groups on one hind wing and one on the other hind wing; two specimens (nos. 10, 22) have four groups on each front wing and two on each hind wing; six specimens (nos. 13, 14, 21, 23, 24, 28) have four groups on each front wing and three on each hind wing; ten specimens (nos. 7, 27, 31, 33 to 37, 41, 42) have four groups on each front wing and four on each hind wing; two specimens (nos. 39, 40) have four groups on each front wing and five on each hind wing; three specimens (nos. 29, 30, 32) have five groups on each front wing and three on each hind wing; two specimens (nos. 2, 4) have five groups on each front wing and four on each hind wing; and one specimen (no. 38) has seven groups on each front wing and four on each hind wing. Of the 15 specimens of butterflies examined it is thus seen that each of 15 has 16 or more groups of pores on both pairs of wings, while of the 28 specimens of moths examined each of 24 has 14 or less groups on both pairs of wings. This indicates that the olfactory pores in butterflies are the more highly developed.

Practically every specimen examined has at least a few scattered pores on the wings, and, as a rule, the fewer the groups of pores the greater is the number of scattered pores. It is common for the pores to extend the full length of one or more veins and to terminate at the distal ends of the veins in pairs as shown in figure 1.

Every leg of the specimens examined bore pores, but the more the legs are reduced in size the fewer the pores they bear. The disposition of the pores on the trochanters and femurs of a few of the species is similar to that of the honey bee, but only occasionally are pores found on the proximal ends of the tibiae and

never on the tarsi, as observed in the Hymenoptera. A few pores, usually near the distal ends of the tibiae, were seen in 21 of these specimens (nos. 1, 2, 3, 5, 7, 8, 11, 12, 19, 22 to 26, 33 to 35, 37, 38, 40, 43), and pores were observed in the tibial spines of 12 individuals (nos. 1, 2, 6, 15, 16, 22 to 26, 28, 43). Relative to the isolated pores on the femurs at the position marked *a* in figure 2 E, one pore was found at this position in 28 specimens (1, 2, 3, 6, 10 to 12, 15 to 17, 19, 23, 26, 28, 30 to 41, 43); two pores were found at this position in three specimens (nos. 5, 13, 14); four pores at this position in one specimen (no. 42).

*c. Generic, specific and sexual variations.* The generic and specific variations are considerable when the total number of pores is considered. The noctuids (Nos. 4 to 9, table 1, p. 43) well illustrate the generic variation and the three species of *Pontia* (nos. 35 to 37) illustrate the specific variation. The total number of pores of the noctuids vary from 852 to 1422, with a difference of 570 pores, and the species of *Pontia* from 632 to 784, with a difference of 152 pores.

Excluding the females with rudimentary wings, the sexual variation is insignificant. The male each of *Bombyx* (nos. 13 and 14) and of *Sanninoidea* (nos. 19 and 20), the peach-tree borer, has 13 more pores than has the female of the same species.

The male of the tussock moth, *Hemerocampa leucostigma*, was not examined, but the number of pores on the rudimentary wings of the female (no. 10) does not seem to be greatly reduced. The front wings are plainly visible to the unaided eye, but the hind wings are not, and both pairs are nothing less than thick pads in which the veins are not distinct. As in *Bombyx*, three groups of pores (nos. 1 to 3) lie on the dorsal surface of each front wing (fig. 3 A) and one (no. 4) on the ventral surface; nos. 5 and 6 are present on the dorsal surface of the hind wing (fig. 3 B), but no. 7, usually present on the ventral surface of the hind wing, is absent. The scattered pores on the front wings are twice, and those on the hind wings are three times as large as the ones in the groups; ordinarily the scattered pores are little, if any, larger than those of the groups.

Both pairs of wings of the female geometrid moth, *Alsophila pometaria* (no. 16), are invisible to the unaided eye, and are so greatly reduced that the front wing (fig. 3 D) is only about three-fourths as large as the tegula (*Tg*) and no larger than the patagia (fig. 3 C) on the prothorax. The hind wing (fig. 3 E) is about one-third as large as the front wing. No pores are present on the ventral surfaces of the wings, and only two groups lie on the dorsal surface of the front wing and only one on the hind wing;

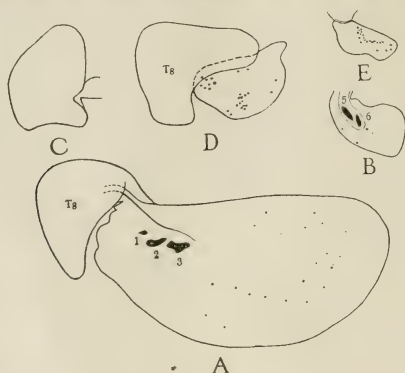


Fig. 3 Wings, tegulae (*Tg*) and patagia of so-called wingless female moths, showing location of groups of olfactory pores and scattered pores on dorsal surfaces of wings. A, tegula and front wing and B, hind wing of tussock moth, *Hemerocampa leucostigma*,  $\times 21$ ; C, patagia, D, tegula and front wing and E, hind wing of the geometrid, *Alsophila pometaria*,  $\times 53$ .

a few scattered pores were also seen on each wing. All of these pores are larger than usual.

To determine the individual variation in the total number of pores, three specimens of the lesser wax moth, *Achroia grisella* (no. 22), were examined. The total numbers of pores on the legs and wings of these moths are 1112, 1168 and 1238. A part of this variation may be attributed to sex, because the sex of each individual was not determined.

The following table (p. 43) includes the family, number and name of the species, the number of olfactory pores on the legs,



TABLE 1

Number of olfactory pores on legs, wings and mouth parts of *Lepidoptera*

| FAMILY          | NUMBER AND NAME OF SPECIES              | NUMBER OF PORES ON |             |            |             | TOTAL NUMBER OF PORES |
|-----------------|---|--------------------|-------------|------------|-------------|-----------------------|
|                 |   | Legs               | Front wings | Hind wings | Mouth parts |                       |
| Sphingidae      | 1 <i>Phlegethontius quinquemaculata</i> | 130                | 453         | 306        |             | 889                   |
|                 | 2 <i>Ceratomia catalpae</i>             | 184                | 502         | 363        | 00          | 1049                  |
| Aretiidae       | 3 <i>Apantesis</i> sp.                  | 138                | 392         | 367        |             | 897                   |
| Noctuidae       | 4 <i>Prodenia arnithogalli</i>          | 95                 | 442         | 478        | 37          | 1052                  |
|                 | 5 <i>Lycophotia margaritosa</i>         | 105                | 500         | 545        |             | 1150                  |
|                 | 6 <i>Agrotis unicolor</i>               | 107                | 485         | 448        | 4           | 1044                  |
|                 | 7 <i>Cirphis unipunctata</i>            | 83                 | 662         | 663        | 14          | 1422                  |
|                 | 8 <i>Alabama argillacea</i>             | 74                 | 432         | 499        |             | 1005                  |
|                 | 9 <i>Caenurgia erechtea</i>             | 127                | 387         | 338        |             | 852                   |
| Liparidae       | 10 <i>Hemerocampa leucostigma</i> ♀     | 160                | 318         | 238        | 00          | 716                   |
| Lasiocampidae   | 11 <i>Tolyte velleda</i>                | 188                | 336         | 366        |             | 890                   |
|                 | 12 <i>Malacosoma americana</i>          | 143                | 389         | 344        | 8           | 884                   |
| Bombycidae      | 13 <i>Bombyx mori</i> ♂                 | 240                | 433         | 401        | 00          | 1074                  |
|                 | 14 <i>Bombyx mori</i> ♀                 | 218                | 423         | 420        | 00          | 1061                  |
| Geometridae     | 15 <i>Alsophila pometaria</i> ♂         | 126                | 284         | 198        |             | 608                   |
|                 | 16 <i>Alsophila pometaria</i> ♀         | 125                | 52          | 45         |             | 222                   |
|                 | 17 <i>Synchlora aerata</i>              | 134                | 278         | 128        |             | 540                   |
| Psychidae       | 18 <i>Thyridopteryx ephemeraeformis</i> | 195                | 262         | 241        |             | 698                   |
| Aegeriidae      | 19 <i>Sanninoidea exitiosa</i> ♂        | 156                | 468         | 379        | 30          | 1033                  |
|                 | 20 <i>Sanninoidea exitiosa</i> ♀        | 146                | 398         | 445        | 31          | 1020                  |
| Pyralidae       | 21 <i>Loxostege obliteralis</i>         | 120                | 466         | 416        |             | 1002                  |
|                 | 22 <i>Achroia grisella</i>              | 141                | 496         | 539        | 59          | 1235                  |
|                 | 23 <i>Ephestia cautella</i>             | 166                | 486         | 634        | 14          | 1300                  |
| Pterophoridae   | 24 <i>Oxyptilus tenuidactylus</i>       | 135                | 272         | 284        |             | 691                   |
| Olethreutidae   | 25 <i>Laspeyresia pomonella</i>         | 147                | 476         | 376        | 14          | 1013                  |
|                 | 26 <i>Eucosma scudderina</i>            | 159                | 432         | 242        |             | 833                   |
| Yponomeutidae   | 27 <i>Atteva aurea</i>                  | 71                 | 507         | 402        | 14          | 994                   |
| Tineidae        | 28 <i>Tinea pellionella</i>             | 138                | 384         | 354        |             | 876                   |
| Nymphalidae     | 29 <i>Argynnis cybele</i>               | 168                | 281         | 182        |             | 631                   |
|                 | 30 <i>Grapta interrogationis</i>        | 113                | 337         | 208        |             | 658                   |
|                 | 31 <i>Euvanessa antiopa</i>             | 73                 | 340         | 216        | 34          | 663                   |
|                 | 32 <i>Junonia coenia</i>                | 104                | 269         | 198        |             | 571                   |
| Lycaenidae      | 33 <i>Heodes hypophlaeas</i>            | 160                | 224         | 168        | 00          | 552                   |
|                 | 34 <i>Everes comyntas</i>               | 178                | 206         | 130        |             | 514                   |
| Pieridae        | 35 <i>Pontia monuste</i>                | 134                | 298         | 200        |             | 632                   |
|                 | 36 <i>Pontia protodice</i>              | 166                | 390         | 228        |             | 784                   |
|                 | 37 <i>Pontia rapae</i>                  | 167                | 301         | 194        |             | 662                   |
|                 | 38 <i>Callidryas eubule</i>             | 134                | 404         | 228        |             | 766                   |
|                 | 39 <i>Eurema nicippe</i>                | 113                | 312         | 202        | 4           | 631                   |
|                 | 40 <i>Eurema euterpe</i>                | 125                | 258         | 206        |             | 589                   |
| Papilionidae    | 41 <i>Papilio polyxenes</i>             | 161                | 350         | 218        | 21          | 750                   |
|                 | 42 <i>Papilio troilus</i>               | 196                | 331         | 160        |             | 687                   |
| Hesperiidae     | 43 <i>Eudamus proteus</i>               | 122                | 248         | 220        |             | 590                   |
| Variation ..... |   | 71—                | 52—         | 45—        | 00—         | 222—                  |
|                 |   | 240                | 662         | 663        | 59          | 1422                  |

wings, mouth parts, and the total number of pores on each of the 43 specimens examined. In the preceding pages the insects are usually referred to in this table by their respective numbers. The blank spaces in the fourth column mean that the mouth parts were either missing or so badly mutilated that the pores on them could not be counted. Owing to the rudimentary condition of the mouth parts, no attempt was made to identify accurately the various mouth appendages, but most of the pores recorded were found on the bases of the palpi, as is best illustrated in the lesser wax moth (no. 22). The only pores found on the mouth parts of *Euvanessa antiopa* and *Papilio polyxenes* (nos. 31 and 41) lie in two groups at the base of the proboscis, on the dorsal surface, in the same position as recently stated for the honey bee by the writer ('16).

### *Structure*

The preceding pages deal with the disposition of the olfactory pores, and a discussion of the anatomy of these organs is given in the following pages.

*a. External structure.* When the superficial ends of the olfactory pores are examined under a high-power lens with a strong transmitted light, the pores appear as small bright spots, each of which is surrounded by darker chitin, the pore border (fig. 4 E, *PorB*) and by the pore wall (*PorW*). The pore aperture (*PorAp*) is usually oblong, but may be round: its size depends upon the focusing level of the microscope, showing that it is funnel-shaped. The size of the pores vary considerably, as may be seen by referring to figure 4.

*b. Internal structure.* The olfactory pores have been called dome-shaped organs, but the domes are not always present as is shown in figures 5 C and 8 C; in those sections in which the domes are not visible the microtome knife probably passed through the organ too far from the pore aperture. The domes (fig. 5 B and D, *D*) in the wings and legs of moths, and in the wings (fig. 9 A) of a butterfly rise slightly above the surface of the surrounding chitin, while in the legs of the same butterfly

the domes (fig. 9 B, D) lie below the surface of the surrounding chitin. Neither Guenther ('01) nor Freiling ('09), who have studied the anatomy of these organs, saw the sense fibers pass through the domes, and consequently they speculated about the function of these pores. Owing to the small size of the pore apertures and to the great thickness of the domes the present

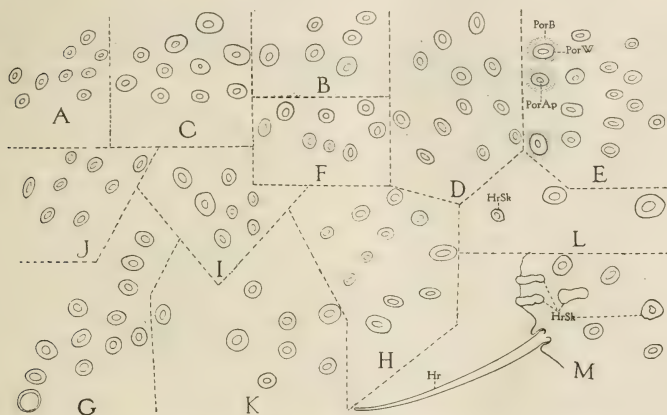


Fig. 4 External view of olfactory pores of *Bombyx mori* ♀, showing variations in size,  $\times 320$ . A, group no. 1; B, 6 of group no. 2; C, 10 of group no. 3; D, 12 of group no. 4; E, 15 of group no. 5; F, 8 of group no. 6; G, group no. 7; H, group no. 8; I, group no. 9; J, group no. 10; K, group no. 11; L, group *a* and a hair socket (*HrSk*); and M, a hair (*Hr*), 4 hairs sockets and 4 pores from tip of hind wing on ventral side. *Hr*, hair, called *S. trichodea* on antenna; *HrSk*, hair socket; *PorAp*, pore aperture; *PorB*, pore border; *PorW*, pore wall.

writer has not been able to find a pore which well illustrated the sense fiber running into the pore aperture, nevertheless four pores (figs. 5 A and D, 8 A and E) were found, each of which had a light streak passing through the dome (D). A few sections cut obliquely clearly show the sense fibers (figs. 7 and 8B) connecting with the pore apertures, so that in the present writer's opinion there can be no doubt about the peripheral ends of the sense fibers coming in direct contact with the external air.

All the olfactory pores studied are more or less flask-shaped structures, although the width of the flask is often equal to its height, and the mouths of such flasks are quite flaring; this is particularly true for the pores in the legs (fig. 5 D and E) of *Bombyx*, but in the wings (fig. 5 A and B) of the same insect the pores are more flask-shaped.

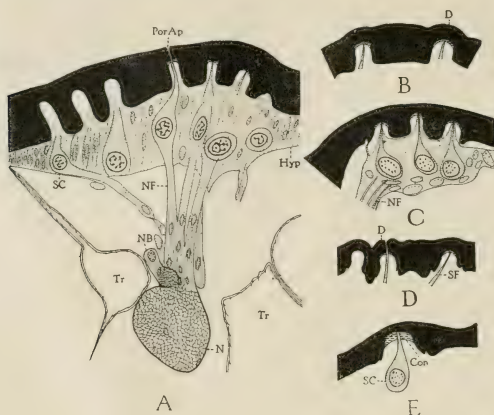


Fig. 5 Cross sections, showing internal anatomy of olfactory pores of *Bombyx mori*,  $\times 506$ . A, from front wing; B, from hind wing, showing dome (D); C and D, from trochanter; and E, from femur. Con, chitinous cone of olfactory pore; D, dome; Hyp, hypodermis; N, nerve; NB, nerve branch; NF, nerve fiber; PorAp, pore aperture; SC, sense cell; SF, sense fiber; Tr, trachea.

Cones are usually present and are of three types: The external boundary of the most common type (fig. 5 E, Con) is convex, that of the second type (fig. 8 C) is almost flat, and that of the third type (fig. 5 A) is concave. The first type is found both in the legs and wings while the other two types are found in only the wings; only the first and second types are present in the wings of *Achroia*, the second type being the more common.

The sense cells (fig. 5 A and E, SC) are more spherical and therefore less spindle-shaped than those found hitherto in other

insects. The nerve fibers (fig. 5 A, *NF*) run directly to the nerves which lie surrounded by blood and the tracheae (*Tr.*) The sense fibers (fig. 5 D and E, *SF*) are easily traced to the cones, but seldom through them. Sometimes they are surrounded by

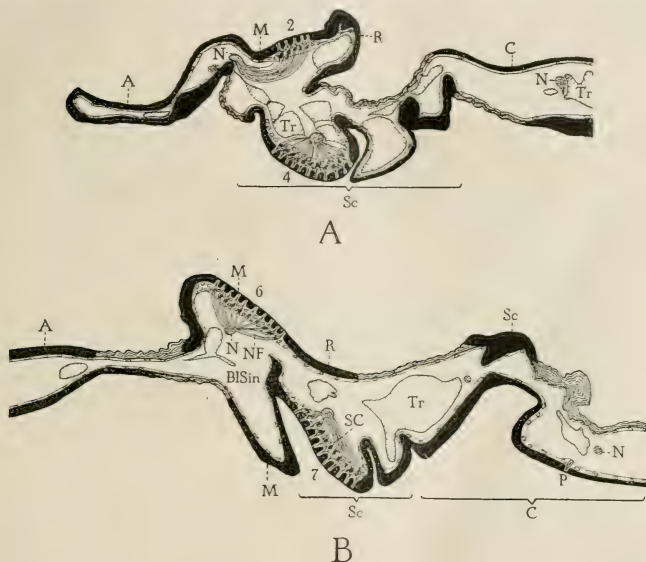


Fig. 6 Semidiagrammatic drawings of cross sections of wings of *Bombyx mori*, showing internal anatomy at bases of wings and groups nos. 2, 4, 6 and 7 of olfactory pores,  $\times 80$ . A, from front wing and B, from hind wing. *BlSin*, blood sinus; *C*, costa; *M*, media; *N*, nerve; *NF*, nerve fiber; *P*, isolated olfactory pore on costa; *R*, radius; *SC*, sense cell; *Sc*, subcosta; *Tr*, trachea.

the hypodermal secretion (fig. 8 A, C and D, *HypS*) which has formed the cones.

To understand the anatomy of the wings at the position where the olfactory pores are located, the reader is referred to figure 6 A and B, which are semidiagrammatic drawings taken from sections across the bases of the wings, A being from the front



and B from the hind wing. At once it is seen that instead of the surfaces of the wings being smooth, as is generally believed, they are more or less corrugated, the elevations being formed by the veins and the depressions usually by the flexible chitin between the veins. The position of a vein on one side of a wing seldom corresponds exactly to the position of the same vein on the other side of the wing, and consequently it is difficult to identify

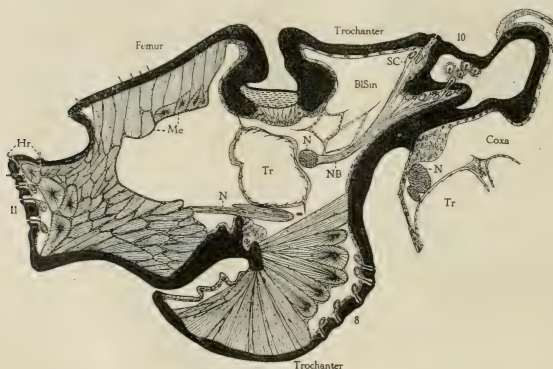


Fig. 7 Semidiagrammatic drawing of an oblique section through femur, trochanter and coxa of *Bombyx mori*, showing internal anatomy at this location in leg, and groups nos. 8, 10 and 11 of olfactory pores, no. 10 being shown partially from a superficial view,  $\times 106$ . *BlSin*, blood sinus; *Hr*, hair, called *S. trichodea* on antenna; *Me*, muscle; *N*, nerve; *NB*, nerve branch; *SC*, sense cell; *Tr*, trachea.

the veins in cross-sections; but after making a study of the serial sections this difficulty was alleviated. The identification of the veins then makes it easy to recognize the groups of pores. The sections illustrated passed through groups nos. 2 and 4 on the front wing and nos. 6 and 7 on the hind wing. The sense cells (*SC*) lie in thickened portions of the hypodermis and are constantly bathed with blood. The nerve fibers (*NF*) spread out fanlike from the nerves and unite with the sense cells. It is to be noted that a nerve (*N*) and one or more tracheæ (*Tr*)

lie in each vein, and that one of the sections passed through an isolated pore in the costa of the hind wing (fig. 6 B, P).

To understand the anatomy of the legs at the position where the olfactory pores lie, the reader is referred to figure 7, which is a semidiagrammatic drawing taken from one section cut obliquely across the femur, trochanter and coxa. In dead insects the femur

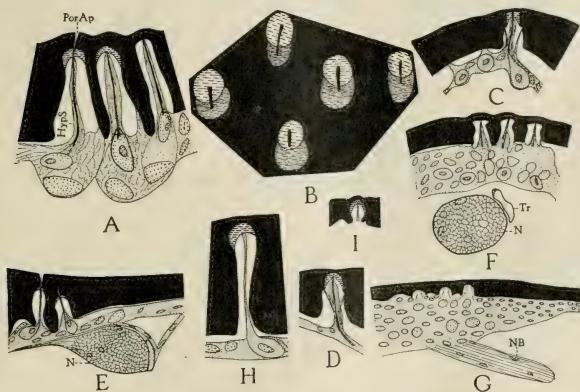


Fig. 8 Cross sections, showing internal anatomy of olfactory pores of other moths,  $\times 506$ . A to G, from wings; H, from trochanter; and I, from femur. A, B and H, from catalpa sphinx, *Ceratomia catalpæ*. B is cut obliquely; C, D and I, from lesser wax moth, *Achroia grisella*; E, from *Prodenia ornithogalli*; F, from *Attēva aurea*; and G, from front wing of tussock moth. *HypS*, hypodermal secretion forming cone; *N*, nerve; *NB*, nerve branch; *PorAp*, pore aperture; *Tr*, trachea.

and trochanter form a right angle with the coxa, and this fact explains how an oblique section may be cut passing through all three segments. This section passed through groups nos. 8, 10 and 11, no. 10 being shown partially in cross section and partially from a superficial view. Most of the muscles (*Me*) are cut longitudinally, but those near the sense cells (*SC*) run transversely, leaving a space, the blood sinus (*BlSin*), in which the sense cells lie. The nerve branch (*NB*) leaves the nerve

(*N*), runs to the hypodermis (figs. 7 and 9 B), and then divides into nerve fibers which unite with the sense cells.

Figure 9 C is similar to figure 6 A, but represents a section cut obliquely through the base of the front wing of a butterfly,

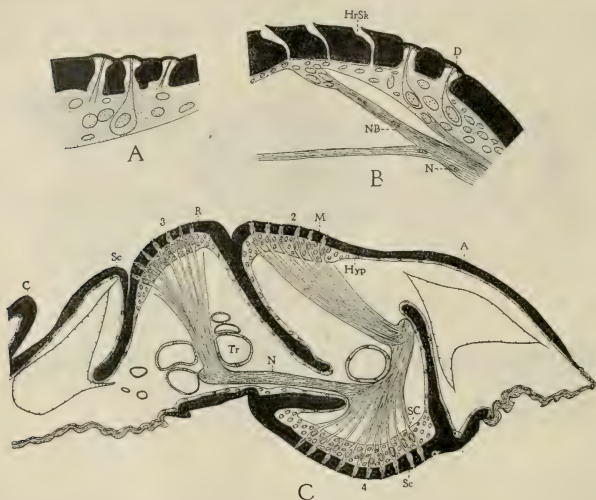


Fig. 9 Cross sections of wing and leg of cabbage butterfly, *Pontia* (*Pieris*) *rapae*, showing internal anatomy of these appendages at positions where olfactory pores are located. A, from front wing and B, from trochanter,  $\times 506$ . C is a semidiagrammatic drawing from an oblique section cut in direction of line *a* in figure 1 A and B, showing groups nos. 2, 3 and 4, of olfactory pores,  $\times 106$ . C, costa; D, dome; *HrSk*, hair socket; *Hyp*, hypodermis; M, media; N, nerve; NB, nerve branch; R, radius; SC, sense cell; Sc, subcosta; Tr, trachea.

*Pontia*, in the direction of the line *a* in figure 1 A and B. The section passed through groups nos. 2, 3 and 4 and longitudinally through the nerve (*N*) for a short distance.

#### THE ANTENNAL ORGANS

Several investigators have studied the morphology of the antennal organs in Lepidoptera, but since Schenk's work ('02)

seems to be the latest and perhaps the best, most of the preceding discussion will be taken from his paper.

### *Disposition*

Schenk has carefully studied the antennal organs in both sexes of the following moths: One geometrid (*Fidonia piniaria*), two bombycids (*Orgyia antiqua* and *Psyche unicolor*), and one zygaenid (*Ino (Atychia) pruni*). He found the following five sense organs on the antennae of both sexes: (1) Pit pegs (*Sensilla coeloconica*), (2) pegs (*S. basiconica*), (3) end pegs (*S. styloconica*), (4) bristle-like hairs (*S. saetica*) and (5) ordinary hairs (*S. trichodea*). In regard to the disposition of the above five types of sense hairs on the feathered antennae of the males and on the non-feathered ones of the females, the following may be said: These sense organs not only lie on the shafts of both types of antennae, but also on all the barbs of the male antennae. The total number of sense hairs of each type found on the antennae of the four moths examined by Schenk is tabulated as follows:

TABLE 2  
*Number of sense organs on antennae of moths (after Schenk)*

| TYPE OF SENSE ORGAN     | FIDONIA  |        | ORGYIA   |        | PSYCHE   |        | INO      |          |
|-------------------------|----------|--------|----------|--------|----------|--------|----------|----------|
|                         | Male     | Female | Male     | Female | Male     | Female | Male     | Female   |
| Pit pegs.....           | 350      | 100    | 600      | 75     | Numerous | 0      | Numerous | Numerous |
| End pegs.....           | 22       | 16     | 50       | 30     | 0        | 0      | 0        | 0        |
| Bristle like hairs..... | 117      | 105    | 80       | 42     | Present  | 0      | 120      | 120      |
| Ordinary hairs          | Numerous | Scarce | Numerous | Scarce | Numerous | 0      | Numerous | Scarce   |
| Pegs.....               | 0        | 5      | 0        | 0      | 0        | 0      | 0        | 0        |

Hicks ('59) and Hauser ('80) saw the same organs, particularly the pit pegs, in the antennae of the butterflies, *Argynnis* and *Vanessa*. Hauser says that each segment of the antennal knob of *Vanessa* carries about 50 pits bearing pegs.

It is thus seen that in Lepidoptera pore plates, common to most insects, are entirely absent, while the pegs are practically wanting; however the pegs seem to be replaced by the end pegs. Of the five sense organs on the antennae of Lepidoptera, only the pit pegs and end pegs are regarded as olfactory organs. Since the end pegs are totally absent in *Psyche* and *Ino*, it does not seem reasonable that they can be olfactory organs for any lepidopteron, and, providing, there is no sexual difference in the size of the pit pegs, the male of *Fidonia* smells three and one-half times as well as the female of the same species; the male of *Orgyia* eight times as well as the female of that species, and while the male of *Psyche* smells well, the female of the same species can not smell at all. It also seems doubtful that the pit pegs can function as olfactory organs.

Now let us inquire if any of these organs are really adapted anatomically to receive olfactory stimuli.

### *Structure*

The ventral side of the shaft (fig. 10 A) of the antenna of *Fidonia* bears more sense organs than does the dorsal side, and the terminal segment bears more pit pegs (*PPg*) than does any other segment. Observed from the side under a high-power lens, the pit pegs look like pits lined with hairs, but when viewed from the tops of the pits, the organs resemble small wheels in that the hairs form the spokes and the base of the peg the hub. Two of the pit pegs thus viewed are shown on the segment third from the last one.

A longitudinal section of a pit peg shows that a peg (fig. 10 B, *PPg*), surrounded by a crown of pseudo-hairs (*Hp*), arises from the bottom of the pit and that the base of the peg is connected with a sense cell group (*SCG*). The end pegs (fig. 10 C, *EPg*) are nothing more than short, stubby hairs supported on stout projections, called styles (*St*), which are innervated. The bristle-like hairs (fig. 10 D), ordinary hairs (fig. 10 E, *Hr*) and pegs are also innervated.

Other authors who have studied the anatomy of these organs describe them as similarly to the preceding account, except the



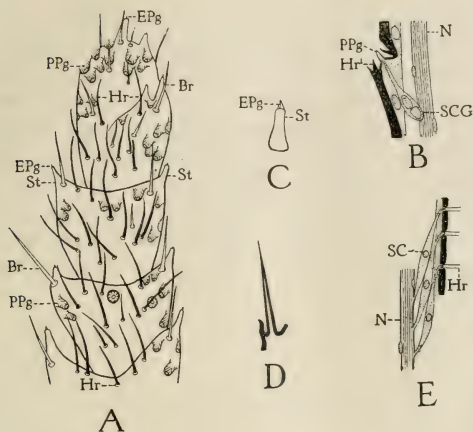


Fig. 10 Antennal organs of the geometrid moth, *Fidonia piniaria*, copied from Schenk ('02). A, ventral view of terminal segment of male antenna, showing disposition of pit pegs (*PPg*), end pegs (*EPg*), supported on the styles (*St*), bristlelike hairs (*Br*) and ordinary hairs (*Hr*) in solid black,  $\times 145$ ; B, longitudinal section through a pit peg,  $\times 570$ ; C, two end pegs, supported on a style,  $\times 220$ ; D, section of a bristlelike hair,  $\times 220$ ; and E, longitudinal section of three ordinary hairs, one-half schematic. *Br*, bristlelike hair (Sensilla saetica) on antenna; *EPG*, end peg (S. styloconica) on antenna; *Hr*, hair, called S. trichodea on antenna; *Hr*<sup>1</sup>, pseudo-hair in pit peg; *N*, nerve; *PPg*, pit peg (S. coeloconica) on antenna; *SC*, sense cell; *SCG*, sense cell group in antenna; *St*, style supporting end peg.

pit pegs and end pegs vary slightly in structure. The pit pegs sometime consist of compound pits instead of single pits, and the shape of the styles supporting the end pegs vary more or less.

Since the peripheral ends of the sense fibers are covered with the hard chitin forming the walls of all the antennal organs, it does not seem reasonable that the outside air carrying odoriferous particles can pass through the chitin in order to stimulate the nerves within.

## SUMMARY

The disposition of the olfactory pores of Lepidoptera is similar to that of Hymenoptera and Coleoptera, but resembles the former more closely. The structure of these pores is also similar to that of those found in other insects, but differs slightly in three following respects: The external ends of those in Lepidoptera are dome-shaped and the only other dome-shaped ones found by the writer are in the lady-beetle, *Epilachna borealis*. The external boundary of the cones may be convex, almost flat, and concave, but hitherto it has always been convex. The sense cells are more spherical than usual.

Compared with the antennal organs, the olfactory pores are better adapted anatomically to receive olfactory stimuli, because the peripheral ends of their sense fibers come in direct contact with the external air, while those in the antennal organs are covered with hard chitin.

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# ON THE PHYSIOLOGY OF THE NUCLEOLI AS SEEN IN THE SILK-GLAND CELLS OF CERTAIN INSECTS

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NINE FIGURES (TWO PLATES)

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## I. INTRODUCTION

The peculiarly shaped nucleus and the unusually large number of the nucleoli in the cells of silk-glands of certain insects have attracted the attention of previous workers on the histology of these organs, but detailed cytological work done in connection with the process of silk secretion is rather limited.

Marshall and Vorhies ('06) published a comparative study of the cell-structure of the silk-glands of *Platyphylax designatus*, a caddis-worm, under different physiological conditions; Vorhies ('08) demonstrated the fact that the multiple nucleoli are derived from a single nucleolus of the ordinary type in the nucleus of the same insect, and noted the increase of the nucleolar material with the growth of the nucleus. These two works have shown that the nucleoli may take some important part in the metabolism of the silk-gland cell, but the exact rôle they

play has remained unsuggested until the appearance of Maziar-ski's ('11) paper, in which he stated that the nucleoli migrate in certain forms into the cell-body, and he regarded these as giving the material for the silk secretion.

Considering the cytological aspect of the phenomenon of secretion in general, the cases in which the secretion granules are derived from the nucleoli are very few, if any. Such authors as Garnier ('00), for the salivary gland of the rat, Maximow ('01), for the similar gland of the dog, claim that the migrated 'Nucleolekörper' are metabolized into secretion products, but this theory does not seem to be as yet satisfactorily established. The observations on the nucleolar migration by Carlier ('99, '06) in the stomach and liver cells of the newt, by Page and Walker ('08) in the mammalian nerve cells, by Walker and Embleton ('08) in the cells of *Hydra*, and by Walker and Tozer ('09) in vegetative cells of various plants and animals seem opposed to the view that the nucleolar material can be considered playing a rôle in the formation of the secretion substance. On the other hand, although not completely established, there is a considerable body of evidence that the chondriosomes, which are widely distributed in the cells of secretory nature, give rise to the secretion granules, as shown by Arnold ('05), Hoven ('10, '11), and Schultze ('11).

In view of the fact that the nucleoli are now generally considered as passive by-products of the nuclear activity, and that, so far as the more reliable of previous observations indicate, the active rôle of the nucleoli in the formation of the secretion products is much to be doubted, it has seemed very advisable to study the phenomenon of the migration of this nuclear element in the silk-gland cells of some insects in greater detail.

The present paper gives a condensed account of my observations, both morphological and chemical, upon the subject. Before proceeding further, I wish to express my sincere thanks to Professor William A. Riley for his stimulating encouragement and valuable advice rendered me during the progress of this work.

## II. MATERIAL AND METHODS

As the material for this study, I used the larvae of the common cabbage butterfly, *Pieris rapae* Linn., and those of a large caddis-fly, *Neuronia postica* Walker.

Usually the silk-glands were taken out by the decapitation method before being placed in the fixing agent; sometimes a dissection in normal salt solution was made and the silk-glands obtained by this method also proved to be as good material. Some other specimens, especially of very young stages, were fixed in toto. Flemming's chromo-aceto-osmic (strong formula) and Hermann's platino-aceto-osmic mixtures were used with good result for fixing the material. Gilson's mercurio-nitric also proved to be a very good fixative. Sections were cut from 3 to 5 micra thick, and the majority of them were stained on the slide with Ehrlich's triacid or Flemming's triple stains. A number of other stains were tried to bring out the changes in the staining reaction of the migrating nucleoli.

## III. OBSERVATIONS AND CONSIDERATIONS

A. *Morphological*

*a. Pieris rapae* Linn (Plate 1). The silk-glands of Lepidoptera have been studied by Helm ('76), Van Liadth de Jeude ('78), Joseph ('80), Carnoy ('84), Blanc ('89), Gilson ('90), Tanaka ('11) and Ito ('15) from different standpoints. More cytological are the papers of Korschelt ('96, '97) and Meves ('97), and especially the interesting cytological paper on the process of silk-secretion by Maziarski ('11).

In his earlier work, Korschelt ('96) noticed two stainable materials within the nucleus, and called them macrosomes and microsomes. He described the former as large round, irregularly angular, or spindle-shaped bodies, and the latter as small particles which always stained darker than the former. The macrosomes, according to this view, represent the chromatin, and the microsomes are nothing but the nucleolar material.



However, Meves ('97), employing various stains, has shown that the microsomes are chromatin and the macrosomes are to be considered as nucleoli, and, although Korschelt ('97) adhered to his previous view in his later paper, the results of subsequent researches by Flemming ('97) and Henneguy ('04) seem to support the view of Meves.

Maziarski ('11) in the silk-gland cells of different lepidopterous larvae, observed that the nucleoli migrate from the nucleus into the cell-body, either as separate bodies or in the form of droplets of 'prosécrét,' being dissolved within the nucleus and accumulated in vacuoles. This, although he does not give any evidence except that this material and the silk-fiber in the lumen of the gland show similar reaction to certain stains, he regards as the source of the silk-secretion.

I found the following conditions in the silk-glands of larvae of *Pieris rapae*. In the larva just hatched (figs. 1 and 2), the nuclei of the silk-glands are more or less round, showing almost no sign of branching. Such nuclei contains a number of the nucleoli and chromatin granules. The nucleoli are of varying sizes and some of them show such a shape as to suggest that they are in the process of division. They are scattered in the nucleus or sometimes grouped together into a loose mass. Not rarely one or two of them lie close to the border of the nucleus. The chromatin granules are imbedded in the linin reticulum. The nuclear membrane can easily be demonstrated in most cases. However, at places where the nucleoli are approaching closely to the border of the nucleus, it has been impossible for me to detect a nuclear membrane.

The cytoplasm is homogenous; but granules of different sizes are to be noted more or less surrounding the nuclei. While most of the granules are somewhat scattered, I find a few almost always occur at the outer border of the nucleus, or contiguous to the nuclear membrane, when this is demonstrable. Now, taking into account the facts of the close approach of the nucleoli to the border of the nucleus and of the partial disappearance of the nuclear membrane, we can conceive that a portion of the nucleoli migrate from the nucleus into the cell-body, forming

the granules we have just observed. The granules of the migrated nucleoli increase in amount as the development advances, and, in a little older larva, the entire of the cytoplasmic area is filled with the granules (fig. 3).

In the larva a little older than those considered above (fig. 4), a mass of the secreted substance occurs in the lumen of the gland. It is apparent, therefore, that the gland is already functional at this stage. Here the nucleus begins to show its branching, and the amount of the chromatin and of the nucleolar material seem to have been increased. The process of the multiplication of the nucleoli is apparently proceeding rapidly. Along the border of the nucleus and the cell-body are many masses of the nucleolar material, indicative of the rather rapid process of nucleoli migration occurring (fig. 5).

The cytoplasmic area shows some elongate vacuoles of varying sizes (fig. 4). Gilson ('90), working on the silk-glands of various lepidopterous larvae, found vacuoles present in the cell-body and also in the nucleus of the cell, and he interpreted them as containing secretory material. Matheson and Ruggles ('07) stated in their work on the similar glands of *Apanteles glomeratus* that "numerous vacuoles are present in the cytoplasm, becoming most abundant during the time of glandular activity," and that "the contents of the vacuoles remain unstained by any of the coloring agents used." Such vacuoles have been observed also in the silk-glands of caddis-worms, not only by Gilson ('96), but also by Marshall and Vorhies ('06), but the latter authors do not think that they contain secretory material. Their observations would show that the cytoplasm in the normal gland cell "presents an appearance free from vacuoles," while after the activity of the gland for two and one-half hours, "a number of fairly large vacuoles are seen along the outer margin of the cell, and such vacuoles persist in the cell-body of the gland which has been active for longer periods (at least up to 250 hours)."

It is rather inconceivable that the amount of the secretion material (supposed to be contained in the vacuoles) in the cell increases, instead of decreases, after the cell has discharged.

and that the normal gland cell, which must naturally be loaded preparatory to discharging, contains no 'secretion material.' These together with the fact that the vacuoles contain no stainable material seem to make Gilson's opinion untenable.

According to Maziarski's ('11) observations, at the height of the secretory activity of the cell, the nucleolar material is discharged from the nucleus, not only as separate bodies, but also as accumulations in vacuoles in the form of droplets of 'prosécrét,' being dissolved within the nucleus. I have gone over my slides with special attention to this point, but the result was negative. The appearances shown in his figures 13 to 27 do not occur in any of my slides, except those that apparently show artificial conditions. I am, therefore, rather doubtful as to whether the conditions observed by Maziarski, which led him to conclude that on certain occasions the nucleoli become dissolved within the nucleus, and carried by vacuoles to the cell-body, were normal. Even if they be normal conditions, we should think that they indicate phenomena that occur very rarely.

The granules of migrated nucleoli appear to be somewhat reduced in number, and some of them begin to show more or less elongated masses at this stage. These granules may naturally be supposed to have something to do with the secretion of the cell, since Gilson's 'vacuole-theory' is to be discarded, and as far as my observations go, there are no other special granules to be detected in the cell-body. Maziarski ('11) claims that basophile granules, which he interprets as derived from chromatin, are also present in the cytoplasm. I have observed such granules only in the degenerating glands obtained from prepupae!

Maziarski ('11) considers the migrated nucleoli as the source of the secretion of the cell, because these and the silk materials in the lumen of the gland show similar color reaction. The fact of the increase in their amount preparatory to the discharging of the cell and its reduction as the cell discharges, also suggest that the granules derived from the nucleoli may be the material source of the silk-secretion.

As the stage advances, the nucleus shows more and more the feature of branching; its contents, both the nucleoli and the chromatin, are apparently being increased in amount with it. The migration of the nucleoli is to be demonstrated as occurring very frequently.

*b. Neuronina postica*, Walker (Plate 2). The silk-glands of caddis-worms have been discussed by Carnoy ('84), Gilson ('96) and Vorhies ('05) to a certain extent. It remained, however, for Marshall and Vorhies ('06) to attempt a minute comparative study of the gland-cells under different physiological conditions. Quoting from their conclusions, Marshall and Vorhies found that:

1. The even optical structure of the cytoplasm in the cells of most normal glands become decidedly changed after activity.

2. The activity of the gland causes the membrane on that surface of the nucleus which lies nearest the outer margin of the gland to become irregular, the most noticeable feature of its irregularity being the pointed processes extending into the cytoplasm.

3. This nuclear membrane may often become indistinct.

4. The secretory activity does not cause the nuclei to become swollen.

5. No plasmasoma or other structure is formed in the nucleus during secretion.

6. As a result of activity the 'nucleolus' becomes very irregular in shape.

As has been said in the preceding section, there has been a considerable difference of opinions as to the nature of the two stainable materials in the nucleus of the silk-gland cells of insects, since Korschelt ('96, '97) insists that the larger granules are the chromatin, and nucleoli are represented by smaller particles, while Meves ('97), Flemming ('97), Henneguy ('04) and Marshall and Vorhies ('06) express opinions that are exactly opposite to that of Korschelt.

Vorhies' ('08) work on the development of the nucleus has finally settled the question in favor of the view that the nucleoli are represented by smaller particles, and at the same time, his studies enabled him to suggest that the "nucleolar material bears a direct relation to the glandular activity," since he observed the



increase of the material with the growth of the nucleus. He, however, did not enter into a detailed discussion of the matter.

The nucleus contains many nucleoli and chromatin granules. The former are of different sizes, distributed in most cases, fairly evenly throughout the nuclear area and sometimes containing vacuoles. The chromatin granules are very fine, and lie imbedded in the linin reticulum. As has already been said by Marshall and Vorhies, the changes occurring in the nucleus during the secretion are not great, and the nucleus retains the appearance here described for the entire history of the secretory activity of the gland. In the cytoplasmic area, however, some remarkable changes are noted to occur.

Figure 6 shows a cross section of a resting gland, i.e., the gland which has not yet discharged the secretion material, and figure 8 one which has apparently done some discharging. One will notice that the granules of different sizes fill the entire area of the cell-body in the resting gland, while in the case of the other one, the amount of the granules is much reduced.

The granules in question are apparently identical with those in the case of *Pieris*, and we can naturally expect them to be derived from the nucleolar material. Close examination of the nuclei with special reference to the nucleoli indicates that this supposition is not incorrect.

As has been observed by Marshall and Vorhies, the nucleus become very irregular in shape, especially on the side facing the outer margin of the cell. Not infrequently, the condition as shown in figure 9 is to be observed, in which the nuclear membrane apparently has disappeared at some part of the surface of the nucleus, and some of the nucleoli are apparently migrating into the cell-body.

Marshall and Vorhies described the appearance of small dark colored areas scattered irregularly in the cell-body, after glandular activity of five hours. They say that after twenty-four hours of activity, small and somewhat elongated masses of dark colored cytoplasm, running parallel to the vacuoles appeared, while in some cases the masses were also noticed along the inner border of the nucleus. Other very significant statements from their paper are the following:



"In many places, the nuclear membrane is quite difficult to distinguish, being lost in the adjacent cytoplasm; this is especially true in the nuclei along whose outer border the dark cytoplasmic areas are numerous" (after one hundred and twenty hours of activity).

" . . . These (the areas of darkened cytoplasm) are here mostly in close proximity to the outer boundary of the nucleus, but exceptionally present along the inner border of the nucleus" (after an activity of two hundred and forty hours).

Their so-called "darkened areas of cytoplasm" are practically identical with what I consider as the nucleolar material extruded into the cell-body or remaining in the nucleus but ready to migrate. They attempted no explanation of the significance of the appearance of the "darkened areas of cytoplasm." If my interpretation be correct, there is no doubt that ten years ago they observed the phenomenon of the migration of the nucleoli in the silk-gland cell of caddis-worms.

The case of the migration of the nucleoli in the silk-gland cells of caddis-worms supports the theory that the extruded nucleolar material is metabolized into the secretion products, since the granules, which are the migrated nucleoli, are in such large number as to render it rather improbable that they represent a degenerating product, and since, moreover, the quantitative relation of the granules to the different physiological conditions of the cell is such as to be naturally expected for the material prepared for secretion.

*c. Summary.* The foregoing observations on *Pieris* and *Neuronia* seem to justify the following statements as regard the morphological changes of the nucleoli in the silk-gland cells of the insects studied:

1. The nucleoli multiply by division of the preexisting ones, and they increase in amount as the gland becomes more functional.

2. Before the gland becomes functional, a portion of the nucleoli begins to migrate from the nucleus in considerable number into the cell-body.

3. The migration of the nucleoli is continued throughout the entire history of the functional cell.

4. The number of the nucleolar masses in the cell-body decreases after some amount of secretion has been discharged from the cell.

The facts enumerated above seem to lead to the conclusion that the nucleoli constitute at least a part of the source of the secretion products of the cell dealt with.

### *B. Chemical*

Perhaps no one has paid more attention to the staining reaction of the nucleoli and the changes in the reaction after the material has passed out into the cell-body, than Walker and other students of his school.

In their paper on the migration of the nucleoli in the nerve cells of mammals, May and Walker ('08) stained the material with (A) basic fuchsin, followed by methylen blue and Unna's orange tannin, or (B) safranin, followed by methelyn blue and Unna's orange tannin. In method A the nucleoli within the nucleus stained blue or violet. They stain purple or red as they pass through the opening in the nuclear membrane, bright red or pink as they come to lie definitely outside of the nucleus, and, as they travel away from the nuclear membrane, they are always stained pink or red. Using the second method, they observed that the nucleoli within the nucleus stain brilliant scarlet, become reddish orange as they migrate, and turn pale orange or yellow when they are completely extruded into the cell-body. "This suggests strongly," the authors say, "that some important chemical or physical change takes place in the nucleolus when it passes into the cytoplasm."

Walker and Embleton ('08) also made a similar observation on the nucleolus of the cells of *Hydra*, employing the same combinations of stains, as Page May and Walker did.

Working on the nucleoli of the vegetative cells of different plants and animals, Walker and Tozer ('09) have made more general statements as regard the staining reaction of the nucleoli as compared to that of other elements of the cell.

The contents of the nucleolus seem always less susceptible to the basic stain than is the chromatin. While in the nucleus, however, the nucleolar contents show a more basic reaction than the cytoplasm and this tendency remains for some time after it has left the nucleus. But in most cases the extruded nucleolus takes less of the basic and more of the acid stain, until it is quite as deeply or even more deeply colored by it than is the surrounding cytoplasm.

Studying the phenomenon of the migration of the nucleoli in the silk-gland cells of lepidopterous larvae, Maziarski paid no special attention as we might desire, to this sort of changes.

In order to throw some additional light upon this phase of the subject, I stained the secretions of the silk-glands obtained from the larvae of *Neuronia postica* and *Pieris rapae*, with different combinations of the stains. From this I got results similar in the main to those obtained by Walker and others in the case of various other cells.

| STAINS  | COLOR OF THE<br>NUCLEOLI WITHIN<br>THE NUCLEUS | COLOR OF THE<br>MIGRATED NUCLEOLI |
|---|--|-----------------------------------|
| Delafield's or Mayer's haematoxylin,<br>eosin.....        | Salmon   | Salmon                            |
| Iron haematoxylin, orange-G.....                          | Black  | Dark orange                       |
| Safranin, licht grün.....                                 | Red  | Greenish                          |
| Borax carmine, blue de Lyon.....                          | Red  | Bluish                            |
| Delafield's or Mayer's haematoxylin,<br>picrofuchsin..... | Salmon   | Pale clay yellow                  |
| Ehrlich's triacid stain.....                              | Pink   | Pink                              |
| Flemming's triple stain.....                              | Red  | Orange                            |

Thus we see that:

(1) The nucleoli within the nucleus are stained more or less energetically by the acid as well as certain of the basic stains, while

(2) The migrated nucleoli always stain with acid stains but they have no or very little affinity, if any, for the basic stains.

In the field of the cellular chemistry, it has been already made known by Miescher, Kossel, Altmann, Hoppe-Seyler, etc., that the 'nucleins' form a series leading downward from the pure

nucleic acid according to the higher percentages of phosphorus and the lesser percentages of albumen contained in those compounds, and the fact that it is the nucleic acid that determines the staining of the nuclear substance is shown by Lilienfeld, Kossel, and others (Wilson, '00, Mann, '02, Jones, '14).

In 1893, Zacharias (later, Heidenhain, '94) showed that in staining the preparations of the nucleins containing different amounts of phosphorus, with alcoholic solution of acid-fuchsin and methyl green, the nucleic acid takes a pure green color, but that the nuclein poorer in phosphorus and that poorest in the same element stained bluish violet and pure red respectively.

Heidenhain ('94) applied this to the case of certain granules in the nucleus of the leucocytes and demonstrated that these granules may show different color reactions by combining with or giving off phosphorus, although they are all exactly alike in morphological characters. The very interesting case of the changes in color reactions (as well as in sizes) of chromosomes in the eggs of *Pristiurus*, first described by Rückert ('93) has been beautifully explained by Wilson ('00) in similar manner.

Applying these principles to the case under discussion, and taking into account the interpretations of somewhat similar changes in chromatic bodies by the previous authors, we may say that the nucleoli originally contain some amount of phosphorus, but as they migrate into the cell-body, the phosphorus seems to be given off from their composition. The migrated nucleoli may, therefore, be considered as albuminous granules, almost or entirely free from phosphorus.

Considering the chemical composition of the silk-fiber, we see that this statement on the chemistry of the migrated nucleoli is perfectly acceptable as that of a constituent of the former, provided that there may be some other substance given off, in addition to the nucleoli, for the formation of the silk-fibers.

#### IV. CONCLUSIONS

1. In the silk-gland cells of insects studied, a portion of the nucleoli migrates into the cell-body, and it forms at least a part of the secretion products of the cell.

This shows that *although the nucleolus may originally be a passive by-product of the nuclear activity, it may also take an important part in the secretory activity of the cell in certain cases.*

2. *As the nucleoli migrate from the nucleus, they seem to give off phosphorus to form themselves one of the lowest members of the nuclein series.*

This statement on the chemical change may hold true for the migrating nucleoli in different other cells.

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## PLATES

## PLATE 1

### EXPLANATION OF FIGURES

All the figures are drawn with camera lucida.

*Pieris rapae*, L.

- 1 A cross section of a silk-gland of a larva just hatched.  $\times 250$ .
- 2 A cross section of a silk-gland of a larva just hatched, a part of the cell.  
 $\times 1200$ .
- 3 A cross section of a silk-gland of a little older larva.  $\times 250$ .
- 4 A cross section of a silk-gland of a little older larva, representing the condition after a moderate discharge of the secretion.  $\times 250$ .
- 5 A part of the gland-cell of a fairly grown larva.  $\times 1200$ .

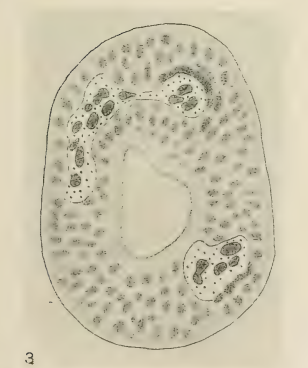
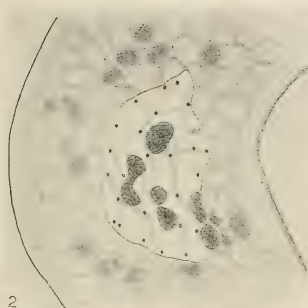
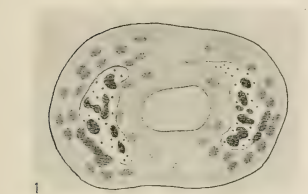


PLATE 2

EXPLANATION OF FIGURES

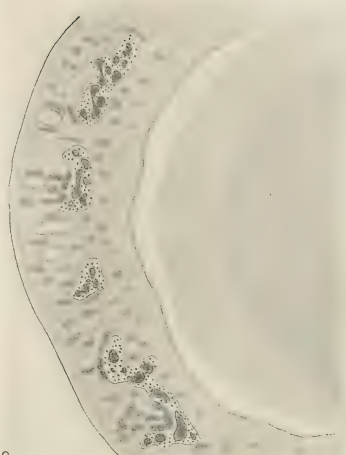
*Neuronia postica*, Walker

- 6 A cross section of a normal silk-gland of a young larva.  $\times 250$ .
- 7 An enlarged portion of a cell shown in figure 6.  $\times 1200$ .
- 8 A portion of a cross section of an active silk-gland of a full grown larva.  
 $\times 250$ .
- 9 An enlarged portion of a cell shown in figure 8.  $\times 1200$ .

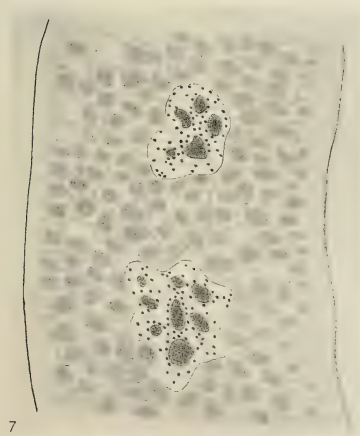




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# THE UROGENITAL SYSTEM OF MYXINOIDS<sup>1</sup>

JESSE LE ROY CONEL

EIGHTY-FIVE FIGURES (TWELVE PLATES)

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## INTRODUCTION

As is well known, Johannes Müller was the first to investigate the urogenital system of the Myxinoids, describing briefly both male and female of *Bdellostoma forsteri* and the European *Myxine glutinosa*. Since his day much has been written regarding this subject. The investigators of the excretory organs include W. Müller ('75), Kirkaldy ('94), Semon ('96), Spengel ('97), and Maas ('97), all of whom worked upon the *Myxine* found in European waters.

Weldon ('84) and Price ('10) are the only ones who have published a description of the excretory apparatus of *Bdellostoma*.

<sup>1</sup> Contribution from the Zoological Laboratory of the University of Illinois. No. 92.

The former investigated *Bdellostoma forsteri*, the latter *Bdellostoma stouti*. Both of these descriptions are confined almost entirely to the structure and probable function of the pronephros, and are comparatively incomplete. Scarcely anything is said about the mesonephros. Price ('96 a, '96 b, '97, '10) has, however, given a detailed account of the development of the excretory system in *Bdellostoma stouti*, which throws much light upon the adult condition. Semon ('96) devotes one paragraph to a comparison between the pronephroi of *Myxine glutinosa* and *Bdellostoma bischofi*.

After J. Müller, Thompson ('59) and Streenstrup ('63) were the next writers upon the generative organs of the Myxinoids, each giving a short account of the mature ova of the European *Myxine*. W. Müller ('75) contributed many points regarding oogenesis in *Myxine* and gave the first description of the minute structure of the testis. In 1886 Cunningham added a few more points regarding the reproductive elements of *Bdellostoma forsteri* and in 1887 he published the first detailed description of the sexual organs and products of *Myxine glutinosa*, introducing the subject of protandric hermaphroditism. Nansen ('87) supplemented Cunningham's description with the results of further investigations, which led him to agree with Cunningham that *Myxine glutinosa* is a protandric hermaphrodite. Cunningham ('92) next contributed a rather lengthy discussion of spermatogenesis in *Myxine*. Ayers ('93) published the first description of the reproductive system of *Bdellostoma stouti*, and soon after Dean ('99) gave an account of the structure of the eggs and development of the embryo. The latest articles which have appeared regarding this subject are those by A. and K. E. Schreiner ('04, '05, '08) which consist of a description of the generative elements of *Myxine glutinosa* and a very thorough discussion of spermatogenesis in *Myxine glutinosa* and *Bdellostoma burgeri*. Besides the foregoing investigators, there are several who have made minor contributions to our knowledge of the genital apparatus in Myxinoids.

The literature contains only a very meagre description of the mesonephros and the reproductive elements of *Bdellostoma* and

practically nothing regarding the urogenital system of the North-American Myxine. The latter animal differs from the European Myxine glutinosa sufficiently to cause some writers to regard the two as different species. Girard ('58) found specific differences "in the external aspect of the snout and buccal aperture, the insertion and proportional development of the tentacles, the form of the body, and . . . the presence of a membranous fin-like expansion along the abdomen," and proposed the name Myxine limosa for the North-American species. Putnam ('73), who compared specimens obtained off the north-eastern Atlantic coast, from the straits of Magellan, and in the Museum of Comparative Zoology, collected off the English coast, however, considers the American animal as merely a variety of Myxine glutinosa. The writer has been unable to obtain specimens of the European animal for comparison. Judging from the literature, there is a great difference in the sizes of the two animals. Putnam's specimens from Liverpool measured  $10\frac{1}{2}$  to 12 inches long (26 to 30 cm.). Nansen, using animals caught near Bergen, and Schreiner, who caught hundreds in Dröbaksfjord, reported the largest adults as 35 cm. long. From Cunningham's account it is to be inferred that the largest adults caught by him off the English coast and near Bergen were from 25 to 42 cm. long, most of them being from 33 to 35 cm., these containing the largest eggs. None of the investigators of the European Myxine report animals longer than 42 cm. The single North-American specimen used by Girard was  $11\frac{1}{2}$  inches long (about 29.25 cm.), and the one examined by Putnam was 12 inches (about 30.5 cm.). The writer has been unable to find in the literature any other measurements of the North-American Myxine. The adult animals used in the present study range from 50 to 79 cm. in length, averaging 62 cm., which is almost twice the length reported for the adult European Myxine. Three specimens, measuring in length 31, 33, and 36.5 cm., respectively, are in a very young stage, the genital ridge being only 2 to 3 mm. wide along its entire course and the eggs being nothing more than dots. Size alone, of course, is not a specific character, but such a great difference as the above is suggestive.



In view of the fact that only two comparatively short descriptions of the pronephros of *Bdellostoma* have been published, and that the mesonephros of *Bdellostoma* and the entire urogenital system of the North-American *Myxine* are still undescribed, it seems desirable:

First, That the pronephros of *Bdellostoma* be re-examined, in order, if possible, to collect additional data which may illuminate some of the uncertain or disputed points in regard to its structure;

Second, That the mesonephros of *Bdellostoma* be described more fully;

Third, That the entire excretory and reproductive systems of the North-American *Myxine* be described and compared with those of *Bdellostoma* and of the European *Myxine*, in order to ascertain whether they present any specific differences or add anything to our comprehension of the urogenital system in *Myxinoids*.

#### MATERIAL AND METHODS

The *Myxine* used in this study consist of 20 specimens which were caught at South Harpswell, Maine, during the summers of 1914 and 1915. Three of the animals are immature females, nos. 7, 17 and 18, measuring 36.5, 33, and 31 cm., respectively. All the other specimens are adults ranging from 50 to 79 cm. and averaging 62 cm. in length. Only one specimen is 50 cm. long and the largest eggs in it are 8 mm. long. The next longest specimen measures 55 cm. and its largest eggs are 22 mm. long.

Twenty-four specimens of *Bdellostoma stouti*, 9 males and 15 females, which were caught at Pacific Grove, California, comprise the *Bdellostoma* material. The smallest is an immature male 33 cm. long. The other males are adults, ranging from 36 to 42 cm. in length, and all the females are adults which range from 34 to 40 cm.

All the animals were killed and preserved in 10 per cent formalin, and later were transferred to 70 per cent alcohol.

The study of the pro- and mesonephros was made from sections stained in Delafield's haematoxylin and eosin. Toto mounts of these organs, cleared in xylol and imbedded in damar, were also used.

The work was done in the Zoological Laboratories of the University of Illinois under the guidance of Dr. J. S. Kingsley. I wish to acknowledge my sense of obligation to Dr. Kingsley for his kindly interest and helpful suggestions, always willingly given.

## OBSERVATIONS

### 1. EXCRETORY SYSTEM

The investigation of the excretory apparatus is based upon *Bdellostoma stouti*, and the results will be presented by describing the organs of this animal and by interjecting, from time to time, comparative notes regarding the same organs in the American Myxine. Unless, therefore, it is specifically stated otherwise, the content of the following discussion refers to *Bdellostoma stouti*.

#### *Pronephros*

The right pronephros lies in the right pericardial cavity immediately dorsal to the portal heart and along the dorso-lateral surface of the alimentary canal, parallel to the dorsal aorta. In figures 1 and 3, which illustrate the position of the right pronephros of *Bdellostoma* and *Myxine*, respectively, in the pericardial cavity, the lateral wall of the cavity has been cut away, but the edges of the pericardium which form the pericardoperitoneal foramen are shown. The pronephros lies along a large diverticulum extending posteriorly from a vein which is called by Jackson ('01) the anterior portal and by Cole ('14) the right anterior cardinal vein. I have had no opportunity to examine injected specimens, but from a study of serial sections the writer agrees with Price ('10) that this vessel is merely a diverticulum with no posterior outlet. In *Myxine glutinosa*, however, Cole ('14) has shown that this vein extends posteriorly after leaving the pronephros and enters the supra-intestinal vein. In a single poorly injected specimen of the North-American *Myxine* the writer found a small twig extending posteriorly from this vein toward the supra-intestinal vein and a larger

branch going to and entering the right postcardinal vein. In *Bdellostoma* the vein varies in diameter from 0.35 by 0.40 mm. to 0.65 by 0.85 mm. at a point about the middle of the pronephros. The vein is always between the two folds of pericardium, by means of which the pronephros is attached to the dorsal aorta and to the wall of the alimentary tract (fig. 5).

The pericardium completely envelopes the pronephros. It encircles each tubule where the latter extends into the pericardial cavity. At the distal end of each tubule the pericardium is continuous with the columnar epithelium which lines the tubule. This enveloping pericardium extends farther down between the tubules in *Bdellostoma* than in *Myxine*.

In all the specimens examined the right pronephros was located in somites 31 and 32, counting the somite in which the eye is located as the first. In only one specimen, a young female, did the pronephros occupy but one somite. In all the other specimens it began in somite 31 and projected into somite 32.

The left pronephros lies in the left pericardial cavity immediately dorsal to the auricle and along the dorso-lateral surface of the alimentary tract, parallel to the dorsal aorta. The left pronephros, also, is connected to the aorta and to the alimentary tract by a fold of pericardium (fig. 5). The line of attachment of this fold of pericardium embraces from one-fourth to one-half of the width, and about three-fourths of the length of the median-lateral surface of each pronephros. In all the specimens the left pronephros was slightly posterior to the right, usually lying in somites 32 and 33. Both right and left pronephroi lie about one millimeter to the right and left of the dorsal aorta, as shown in figures 45 and 46.

The left pronephros lies along a vein extending posteriorly from the left anterior cardinal. From his sections the writer could not trace this vein to any posterior connection in *Bdellostoma*, and concludes, with Price ('10), that it is merely a diverticulum. In *Myxine*, however, the writer found that this vein continues posteriorly and enters the large vein formed by the union of the two posterior cardinals (fig. 4), the left ductus Cuvieri of Cole ('14). Like the right vein, this vein along the

left pronephros lies between the folds of pericardium which attach the pronephros to the dorsal aorta and to the digestive tract. Since no name has been given to these veins, hereafter in this discussion they will be referred to as the right and left pronephric veins. The position of the left pronephros in the pericardial cavity is represented in figures 2 and 4.

In shape the pronephros is never exactly the same in any two specimens nor on both sides of the same specimen. In most cases, however, it is somewhat three-sided, though it is sometimes flat on the attached side only and the remaining surface is rounded. The ends always taper more or less, the anterior end usually being the more pointed. This is due to the fact that at its anterior end the pronephros always invariably consists of only one or two small lobules, while the posterior end is usually composed of several lobules crowded together in a compact mass. Figures 7 to 11 represent pronephroi taken at random and illustrate the variation that may occur in the shape. Figure 11a represents a cross section of a pronephros and illustrates the three-sided form. When three-sided the head-kidney is more or less wedge-shaped, flattened dorso-ventrally with the sharp edge of the wedge opposite the point of attachment. In this condition the pronephros has the appearance of having been subjected to pressure, probably due to the distended portal heart and auricle pushing it against the dorsal body-wall.

The size of the pronephros is also quite variable, as the following few measurements will indicate:

| SPECIMEN<br>NUMBER | SIDE  | LENGTH     | SURFACES   |            |            |
|--------------------|-------|------------|------------|------------|------------|
|                    |       |            | Dorsal     | Ventral    | Lateral    |
|                    |       | <i>mm.</i> | <i>mm.</i> | <i>mm.</i> | <i>mm.</i> |
| 5                  | Right | 6.0        | 1.75       | 1.5        | 1.25       |
|                    | Left  | 5.0        | 2.0        | 2.0        | 0.75       |
| 7                  | Right | 5.75       | 1.75       | 1.75       | 0.75       |
|                    | Left  | 5.0        | 1.5        | 1.25       | 0.75       |
| 16                 | Right | 6.0        | 2.25       | 2.0        | 1.0        |
|                    | Left  | 5.0        | 1.75       | rounded    |            |
| 18                 | Right | 5.0        | 2.0        | 2.0        | 1.0        |
|                    | Left  | 7.0        | 2.0        | 2.0        | 0.5        |

It was observed that the right pronephros is usually slightly larger than the left. In none of the specimens was a pronephros found even one-half as large as those of *Bdellostoma forsteri*, as reported by Weldon ('84), namely 20 to 25 by 5 to 7 mm. There is no correlation between the size of the pronephros and the length of the animal in *Bdellostoma*. Weldon does not give the length of the animals examined by him, hence we do not know whether the great difference in the sizes of the pronephroi of *Bdellostoma stouti* and *Bdellostoma forsteri* may be explained by a difference in the length of the two animals. The adult *Bdellostoma* used in the present investigation range from 33 to 42 cm. long, averaging 38 cm. The pronephroi represented by the above table are all from adult animals.

The writer has been able to find only one statement regarding the size or shape of the pronephros in the adult European *Myxine*. W. Muller ('75) mentions that they are 'länglich,' 3 to 4 mm. long. Following is a table of dimensions of a few pronephroi of the North-American *Myxine*:

| SPECIMEN<br>NUMBER | LENGTH OF<br>ANIMAL | SIDE  | LENGTH OF<br>PRONEPHROS | SURFACES   |            |            |
|--------------------|---------------------|-------|-------------------------|------------|------------|------------|
|                    |                     |       |                         | Dorsal     | Ventral    | Lateral    |
|                    | <i>cm.</i>          |       | <i>mm.</i>              | <i>mm.</i> | <i>mm.</i> | <i>mm.</i> |
| 2                  | 58                  | Right | 5.5                     | 2.0        | 2.0        | 0.75       |
|                    |                     | Left  | 5.5                     | 2.0        | 2.0        | 0.75       |
| 6                  | 61                  | Right | 7.0                     | 2.5        | 3.0        | 1.5        |
|                    |                     | Left  | 6.0                     | 2.0        | 2.5        | 1.0        |
| 13                 | 63                  | Right | 8.5                     | 2.5        | 2.75       | 0.5        |
|                    |                     | Left  | 7.0                     | 2.0        | 2.25       | 1.75       |
| 1                  | 63                  | Right | 9.0                     | 4.0        | 4.0        | 2.5        |
|                    |                     | Left  | 8.5                     | 3.0        | 3.0        | 3.0        |
| 3                  | 64                  | Right | 9.0                     | 3.0        | 3.0        | 1.0        |
|                    |                     | Left  | 8.0                     | 2.0        | 2.0        | 1.0        |
| 12                 | 67                  | Right | 6.0                     | 2.0        | 2.0        | 1.0        |
|                    |                     | Left  | 10.0                    | 2.0        | 2.0        | 1.0        |
| 10                 | 68                  | Right | 10.25                   | 1.0        | 4.5        | 4.5        |
|                    |                     | Left  | 8.5                     | 5.0        | 5.0        | 2.0        |

As is indicated by the above tables, the pronephroi of *Myxine* are considerably larger than those of *Bdellostoma*. This is to be expected from the great difference in the size of the adult ani-



mals. The writer found that there is a correlation between the size of the pronephros and the length of the animal in *Myxine*, but not a sufficiently large number of specimens was available to prove this to be constant. The largest pronephroi (those of *Myxine* no. 10) are in an animal which has the appearance of being quite old. The skin is darker than usual, very thick, wrinkled and hard, the muscle fibers in the body wall are large and coarse, the walls of the alimentary canal are very thick, the liver and gall bladder are very large and coarse. In this specimen the pronephroi are a very dark brown color; in all the other specimens and in all the *Bdellostoma* examined the pronephros is gray. It is probable that the size of the pronephroi is more correlated with the age than with the length of the animal. The right pronephros of *Myxine* was almost always larger than the left.

Price ('04) found that at some stage of embryonic development of *Bdellostoma stouti* an excretory tubule appears in each somite, from the 11th to 13th to the 79th to 82d. That is to say, in the embryo pronephric tubules are present in the same segments with gills. As the gills shift posteriorly (Dean, '97) these pronephric tubules are pushed along behind them and eventually become crowded together in a "small, compact body, the pronephros of the adult, in the region a little posterior to the thirtieth segment" (Price, p. 132). Price does not mention anything in regard to the history of the veins connected with the tubules in this movement posteriorly.

As would be expected, there is a limitless variety in the minute structure of the pronephroi. It is well known that each pronephros is composed of a large number of small tufts or lobules of tubules which open separately into the pericardial cavity. Usually there are fewer of these lobules at the anterior than at the posterior end, and one or two lobules may be entirely separated anteriorly from the rest of the pronephros. Figure 12 represents a reconstruction on millimeter paper of the right pronephros of *Bdellostoma* no. 15. This pronephros was cut in transverse sections, each 10 microns thick, and it will be used as a

basis for the description of the minute structure of the head-kidney of *Bdellostoma*, as follows:

*Histology.* The tubules in each of the two most anterior lobules anastomose inwardly and form a single tubule which unites with one similarly formed from the other lobule, and the single tube thus formed constitutes the central duct of the pronephros. The first appearance of the central duct occurs in the 10th section from the anterior end of the pronephros. All of the pronephroi of *Bdellostoma stouti* examined by the writer have a central duct, which is so evident that it can not possibly be interpreted as anything else. Price ('10) found a central duct in the head-kidney of *Bdellostoma stouti*, and Weldon ('84) described one for *Bdellostoma forsteri*. Semon ('96) thought that Weldon was mistaking the venous sinus (pronephric vein) for a central duct, but Weldon's description of the duct coincides so nearly with the condition in *Bdellostoma stouti* that it seems very probable that *Bdellostoma forsteri* has a central duct in the pronephros. In the 10th section from the anterior end of the pronephros the lumen of the duct is 0.02 mm. wide, but it enlarges immediately and is 0.15 mm. at the widest part. The duct proceeds posteriorly, entering the pronephric vein in section 20 and ending blindly in the vein in section 48. From its beginning this short segment of the central duct is surrounded by a mass of large, deeply stained nuclei, which will be referred to hereafter in this discussion as the 'central mass' and which will be described in detail later. This mass is distributed somewhat regularly around the central duct, now more on one side, now more on the other. Some idea of its gross appearance may be had from figure 12. At its widest part the mass measures 0.21 mm. from its outer edge to the lumen of the duct, and it extends 0.14 mm. beyond the posterior end of the duct.

No trace of the central duct is again seen until section 52 is reached. Here the tubules of one lobule unite into a single tube, and the latter forms the beginning of the central duct. The lumen of this segment of the duct is 0.05 mm. in diameter at first, but measures 0.09 mm. at the widest part. The duct proceeds posteriorly to section 60, receiving in its course the tube

from another lobule. In section 60 the duct enters the pronephric vein, and remains in it to section 80 where it ends blindly. From its beginning this segment of the duct is also surrounded by a central mass like the one around the first segment of the duct. In transverse section the mass is circular in outline and is distributed somewhat regularly around the lumen of the duct. At its widest part the mass measures 0.16 mm. from its outer surface to the lumen of the duct. Only two lobules of the pronephros are connected with this segment of the central duct.

The third segment of the duct appears in section 90, and is formed by the main tube of one lobule. In this section the lumen of the duct is 0.05 mm. in diameter, but becomes as wide as 0.20 mm. This segment of the duct extends to section 150 and there breaks up to form the tubules of three lobules. Altogether there are four lobules connected with this segment. The duct runs parallel to the pronephric vein, and up to section 115 is separated from the latter by a wide layer of connective tissue. In this distance one vein 1 mm. wide and another 3 mm. wide permit blood to flow into the pronephric vein from capillaries which run between the tubules of the pronephros. At section 115 the duct widens abruptly and extends downward in the pronephric vein. In the vein the duct becomes sheathed in a central mass which resembles in all respects the masses which surround the first and second segments of the duct. In section 117 the duct opens through the central mass into the lumen of the pronephric vein. This opening is 0.05 mm. wide. Another such opening 0.09 mm. wide occurs in section 250, and a third 0.04 mm. wide in section 320. These openings are not breaks or tears, but are natural apertures lined by a single layer of squamous epithelium which is continuous with the columnar epithelium of the duct on the one hand and with the endothelium of the pronephric vein on the other. Figure 20 represents one of these openings. At least one, often more, such opening connecting the lumen of the central duct with the lumen of the pronephric vein was found in every pronephros of *Bdellostoma* examined. These openings occur only through the central mass, never through the walls of the central duct when not surrounded by the central mass.

To return to the description, the duct remains in the central mass to section 137 where it leaves the vein and breaks up into the tubules of three lobules. The central mass, however, continues in the lumen of the vein independent of the duct, and in section 147 the fourth segment of the central duct appears in it. The central mass projects into the pronephric vein as indicated in figure 12 and has a cylindrical shape. Between sections 137 and 147 it is 0.30 by 0.40 mm. in transverse section.

The fourth segment of the central duct begins as the result of the anastomoses of the tubules of four lobules whose main tubes unite near section 150 and form the central duct. In this section the duct is 0.05 mm. wide. At first the duct lies in the connective tissue at the base of the pronephros, but in section 157 it bends toward and enters the central mass in the vein. Here the lumen of the duct is 0.27 mm. wide. In this segment one side of the duct remains closely applied to the connective tissue at the base of the pronephros and retains its columnar lining, while the other side extends along the central mass to section 200 where it bends toward its fellow, thus narrowing the lumen of the duct considerably, and the segment ends by the duct branching into the tubules of a single lobule. There are eleven lobules connected with this segment of the duct. The central mass continues without interruption in the pronephric vein to section 240 where the fifth segment of the duct enters it. That is to say, the central mass extends from section 200 to section 240 (a distance of 0.40 mm.) entirely disconnected from the central duct. In the region of the fourth segment the central mass resembles in every respect that around the preceding segments of the duct. In the portion between sections 200 and 240, however, the mass changes its shape in transverse section from circular to elongated, measuring 0.15 by 0.55 mm. in the largest portion. Figure 20 represents the shape of the mass at this place. This figure also indicates the relation of the size of the mass to the lumen of the vein. In this particular pronephros the duct and the mass together in the largest parts never occupy more than about one-half of the lumen of the vein as viewed in transverse section. In one specimen, however, the central duct was



so large that it, together with the central mass, almost entirely obstructs the lumen of the vein. Between sections 205 and 230 the pronephric vein opens directly between the tubules as a large sinus (fig. 20), and another such sinus lies between sections 255 and 300. At all other places, however, there is a strip of connective tissue from 0.01 to 0.05 mm. wide, or even wider, between the pronephros and the vein. In one specimen, though, the vein opens directly into the spaces between tubules along the entire base of the pronephros. But even here the pronephros stands upon the vein as illustrated in figure 20, so that in no sense can the head-kidney be said to lie buried within the vein, a misleading statement which occurs very frequently in the literature. Where the vein opens between the tubules as a sinus, the capillaries, which otherwise carry the blood between the tubules, have broken down and only the pericardial sheath and the endothelium of the sinus confine the blood in the intertubular space. These vascular spaces between the tubules are not 'sinusoids' according to Minot's use of the term. The pronephric tubules do not invade the vein, for, as Price ('10) has shown, the branching to form new tubules always occurs in a direction away from the pronephric vein, "just back of the nephrostomes."

The fifth segment of the central duct begins in section 212 where the tubules of one large lobule unite into a single tube which widens to form the duct. The latter extends posteriorly in the connective tissue at the base of the pronephros parallel to the pronephric vein to section 240, increasing gradually in width from 0.04 mm. to 0.15 mm. In this section the duct bends downward so that one side enters the central mass, which still remains in the vein. The wall of the duct which lies toward the pronephros retains its columnar structure, but the wall which lies in the central mass breaks down completely soon after entering the mass and presents a jagged appearance, and the nuclei bordering the lumen have no definite arrangement. In sections 243 to 255 the central duct opens through the central mass into the lumen of the vein. The mass is similar in all respects to that surrounding the preceding segments of the duct. In section 260 the duct divides, one branch entering a lobule and breaking up



into tubules, the other continuing posteriorly in the central mass and ending blindly in it at section 274. In section 276 the duct begins again as a part of the sixth segment, just opposite the blind ending of the fifth segment. There are six lobules connected with the fifth segment of the duct.

The sixth segment of the duct arises in section 273 from the union of the tubules of one lobule. The duct enters the central mass in the vein in section 300, and is here 0.12 mm. wide. A small twig extends anteriorly in the mass to section 276 where it ends blindly and is separated by only one section from the end of the fifth segment of the duct. Undoubtedly at one time the fifth and sixth segments of the duct were connected at this point. The sixth segment extends posteriorly through the central mass to section 380 where it ends as a single tubule which runs out of the vein and opens into the pericardial cavity. In sections 318 to 323 the duct opens through the central mass into the pronephric vein, and in this region the duct and neighboring tubules contain many blood corpuscles. In section 350 the duct leaves the central mass and the columnar cells of the walls of the duct become double their usual length and are thrown into longitudinal folds like those of the mesonephric duct, to be described later. This folded condition of the epithelial walls of the duct continues to section 370; then the cells diminish in length to the normal size for the tubules, and the duct here ends as a single tubule, as explained. There are six lobules connected with the sixth segment of the duct.

The seventh segment of the central duct begins in the central mass in section 340 beside the sixth segment. It continues in the mass, which still lies in the pronephric vein, to section 365 where it leaves the mass and vein and divides into two branches, one of which ends by branching into the tubules of a lobule and two single tubules, the other breaks up into the tubules of the four most posterior lobules of the pronephros. The lobules connected with this segment of the duct are in such a compact mass that they can not be counted with any degree of certainty.

The eighth and last segment of the central duct arises in the central mass in section 370 beside the seventh segment. The

mass continues around the duct to section 380 and there ends, remaining in the lumen of the vein. The duct proceeds posteriorly, and in section 385 divides into three tubules, each of which leaves the pronephric vein and enters the Bowman's capsule of the Malpighian body of the pronephros, opening into the capsule by a nephrostome. There are no lobules connected with the eighth segment of the duct.

Price ('14) suggests that perhaps each lobule of the pronephros represents a single original tubule and its secondary branches. This is scarcely probable, for there is a total of 31 lobules connected with the first six segments of the duct in the above described pronephros, while those of the seventh segment could not be counted with accuracy but there are at least four. If the most anterior original tubule lay in somite 11, then there could be only 21 tubules at most to and including somite 32, the definitive position of the tubules after they have been pushed posteriorly by the gills. In the pronephros under discussion, however, there are at least 35 lobules.

In *Bdellostoma* none of the tubules in any of the pronephroi examined by the writer show any signs of degeneration, and the lumen of each tubule opens into the pericardial cavity at one end and is continuous, either directly or indirectly, with the lumen of the central duct. The lumen of almost every tubule is filled with a coarsely granular coagulum which is more or less shredded into long processes resembling flagella (fig. 16). These processes are attached at one end to the columnar cells, usually one to a cell, and the free end extends inward toward the base of the tubule, that is, toward the central duct. The lumen of the central duct sometimes contains a comparatively small amount of this coagulum. The writer was unable to demonstrate cilia in the pronephric tubules, but it is probable that his material was not suitably preserved to permit these to be seen if present.

The tubules are very simple in structure. Each is a cylinder consisting of a single layer of columnar cells, which are continuous at the mouth with the pericardium and, at the base, with the columnar cells of the central duct. The mouths of the tubules are sometimes funnel-shaped (fig. 13), but are usually constricted

(fig. 14). The columnar cells which constitute the wall of the tubules are approximately the same size in all the tubules, varying somewhat around 0.0135 mm. high by 0.004 mm. wide. The nucleus is always at the base of the cell and occupies the entire width and from one-half to two-thirds its length. At the base of the cells the cell-walls fuse to form a thin basal membrane, the outer limit of the tubule. When connective tissue occupies the space between the tubules, it lies next to the basal membrane, and when a blood sinus fills the inter-tubular space, the endothelium of the sinus usually lies against the basal membrane, completely surrounding the tubule (fig. 15). The lumen is practically the same in diameter in all of the tubules, and the outside diameter of the tubules is likewise approximately the same for all tubules.

The specimens of *Bdellostoma* examined were within a few centimeters of being the same length, and the dimensions of the tubules are about the same in all the pronephroi examined. At the mouth of the tubule the lumen narrows slightly, but otherwise is approximately the same diameter throughout. Often, however, a tubule is constricted in one or two places. The lumen is approximately 0.0216 mm. wide, and the outside diameter of the tubule is about 0.054 mm.

The protoplasm of the columnar cells of each tubule is coarsely granular, and the boundary of the cell facing the lumen is usually marked by many granules and one or two long processes which consist of granules (fig. 16). The nuclei are more or less oval and always contain many large deeply stained bodies. The cells of the tubules are continuous with those of the central duct at the proximal end, and with those of the pericardium at the distal end.

The cells of the central duct are also columnar and are usually longer than those of the tubules. The character of the cytoplasm and nuclei in the duct cells is practically the same as in the tubules, and the basal membrane of the latter is continuous with the basal membrane of the former. The cells of the duct in a given pronephros vary in length at different points, but any increase or decrease is gradual. They may be as long as 0.0324 mm. in

the midregion of the pronephros. The diameter of the lumen of the duct varies widely in an individual specimen. It may be as small as a tubule in places, then increase to several times that width. The duct and its lumen may be continuous throughout the entire length of the pronephros or may be broken up into disconnected segments. The most continuous duct was found in a young male. The duct in this specimen also has the least amount of central mass connected with it, and the mass is almost entirely confined to the posterior end of the duct.

The structure which has been referred to as the 'central mass' was first described by Kirkaldy ('94) for *Myxine*, and the writer has adopted his name for it. It is possible that Weldon's lymphatic tissue may have been the same thing as this central mass, but his short description does not enable us to be positive. This mass of tissue has been the subject of much discussion by investigators of the pronephros of Myxinoids. Weldon ('84) described a mass of lymphatic tissue which he found at the posterior end of the head-kidney of *Bdellostoma forsteri*. He states that the central duct ends posteriorly "in a mass of tissue . . . resembling the trabecular supporting tissue of a lymphatic gland." Into this mass strands of blood vessels pass from the glomerulus which lay beside it. Upon the basis of this lymphatic tissue Weldon thinks the pronephros becomes transferred into and functions as a suprarenal body. Kirkaldy found a difference in the mass, in animals without ova and those with them. In the former the mass was divided into separate parts, and each part was regarded as a glomerulus supplied with a capsule, inside of which was a characteristic loop (his fig. 2). In an animal with ova he found no central duct or glomerulus in the pronephric vein, but their former position was occupied by a mass of small cells with small nuclei and larger cells with large, round, and deeply stained nuclei (his fig. 7). Capillaries were numerous in this mass. Kirkaldy regards the mass as the degenerating central duct of earlier stages, hence Myxinoids may be considered as representing a stage in the phylogenetic reduction of the head-kidney, and the latter may represent the mesoblastic part of the supra-renal bodies.



Semon ('97) regards this mass as the glomeruli of the head-kidney. Eventually, however, the pronephros becomes transformed into a suprarenal body. He found the mass rich in blood vessels.

Spengel ('97) thinks this mass was never glomerular, but that it is either a suprarenal body or a lymph organ, and that it is the result of a metamorphosis of the inner ends of the pronephric tubules.

Maas ('97) thinks the mass differs distinctly from a glomerulus. Blood vessels occupy only a little space in it, and it is greatly dissimilar to tubule epithelium. It resembles stages in the development of the Müllerian duct of higher animals, especially reptiles. It is also not unlike the medulla of suprarenals.

Price ('10) evidently saw this mass, but does not describe it. He states that "in places the wall of the duct may become greatly thickened by an increase in the number of epithelial cells. These change their shape, and become much more loosely arranged, so that the tissue loses entirely the structure of columnar epithelium." Price adds that these thickenings are not supplied with arteries.

In specimens with a duct which is continuous throughout the entire length of the pronephros, the central mass appears only in the posterior third of the pronephros where it surrounds the duct and lies in the pronephric vein. In most of the specimens, however, the duct is broken up into segments which are not connected with each other, and that part of each segment which lies in the vein is surrounded by the central mass. The mass is found only in the pronephric vein (a very small quantity may be connected with the duct immediately before it enters the vein). A typical distribution of the mass is represented by figure 12. It may project into the vein like a glomus, one side remaining in contact with the base of the pronephros, or it may lie in the lumen of the vein, completely surrounded by blood, and may be attached to the base of the pronephros at one end only. The central mass is found only in connection with the central duct and is always attached to the latter, though one end of the mass may extend in the pronephric vein some distance (as much as



40 sections, 0.4 mm.) beyond the end of the lumen of the duct. The mass is more or less cylindrical, though in places it may be considerably widened as if bent upon itself, and then it is elongated in transverse section. In none of the specimens does it entirely fill the lumen of the vein. In those pronephroi in which the duct is almost continuous, however, the lumen is so great in diameter, that the duct, together with the central mass surrounding it, almost entirely fills the lumen of the vein, leaving only a very narrow space between the mass and the vascular endothelium. The endothelium of the pronephric vein is continuous around the mass (figs. 17 and 22), but is here much thinner than when lining the vein, and its nuclei can be observed only now and then. The surface of the mass is smooth and convoluted.

Figure 18 represents a transverse section through the central mass, and illustrates the typical condition in *Bdellostoma*. It is composed of a large number of cells, the nuclei of which are more or less oval in shape. The latter are of various sizes, the largest and most numerous averaging 0.0054 by 0.0108 mm. These nuclei are not distributed in any regular manner, large, small and intermediate sizes are mingled in all parts of the mass. Also, there is no regularity in the manner of their arrangement; some are isolated completely from surrounding neighbors, others are collected in groups of two or three, and again six or eight may be closely crowded together in a mass. There is no definite cellular structure observable in the central mass. Most of the nuclei contain many large, deeply stained granules, but many have only a nuclear membrane with no granules inside. Many of the nuclei have no cytoplasm surrounding them, while others are imbedded in what resembles the granular cytoplasm in the cells of the central duct and tubules. This granular cytoplasmic ground-work is scattered throughout the central mass and, in places, is drawn out into slender processes which resemble those found in the tubules. There is no connective tissue in the central mass. In an occasional transverse section of the mass there are one to three or four giant nuclei, but these are not frequent in *Bdellostoma*. About one hundred sections of the mass were examined before a section containing one of these giant nuclei

was found. Occasionally such nuclei occur in the walls of the duct or of the tubules near the central mass. These giant nuclei are much more deeply stained than are the other nuclei of the mass, and the cytoplasm, which always surrounds them, stains like that of blood corpuscles, but much more deeply. These giant cells are interpreted as greatly enlarged blood corpuscles. They do not have the elongated characteristic shape of the blood corpuscles, but are usually rounded and quite irregular in outline. In the central mass they are surrounded by a very thin membrane which is interpreted as endothelium, although no nuclei were observed in it. In the walls of the duct or tubules, however, nuclei occur in the membrane surrounding the giant blood corpuscles and here it is undoubtedly endothelium. These giant cells are also frequently seen in capillaries which occur in the connective tissue surrounding the mesonephric duct, and here there is no doubt that they are in blood vessels.

Excepting the giant corpuscles just described, the writer rarely found a blood corpuscle or blood vessel in the central mass. An attempt was made to find blood vessels entering the mass, but without success. The blood corpuscles have a distinctly characteristic shape and appearance, and the cytoplasm stains more deeply in eosin than that of any of the other cells in the pronephros, hence the corpuscles are easily discerned in any tissue. There are many spaces in the central mass, but they are not lined by endothelium and never contain blood corpuscles. Furthermore, the writer found no vessels leaving the central mass to enter the vein or any surrounding tissue. Also, natural appertures in the endothelial wall of the mass were diligently but vainly sought; the mass is completely shut off from the blood in the vein. One series of sections is especially well stained to demonstrate this fact. The blood corpuscles are stained a deep, yellowish-red, which is strikingly different from the pale blue of the central mass. The blood corpuscles surround the latter as a dense mass which almost fills the lumen of the vein. On account of the striking contrast in stain, a single blood corpuscle inside the central mass would be instantly recognized if present. But

none was found. Price ('10), also, found no blood vessels in the central mass in *Bdellostoma*.

When not surrounded by the central mass, the central duct retains its columnar walls, but loses them upon entering the mass. The walls break down completely and the cells become scattered in the mass. The lumen of the duct may continue in the mass, but it has no organized lining and is bordered by nuclei and strands of cytoplasmic ground-work (fig. 29). The columnar lining of the central duct is never continuous with the epithelial sheath around the central mass. In view of this fact, it is difficult to regard, as Semon ('96) does, the central mass as a series of glomeruli into whose Bowman's capsules the tubules of the pronephros enter through their 'Innentrichter,' for Semon ('96, '97) himself has shown that, in the mesonephros, the columnar wall of the tubule of a Malpighian body is continuous with the squamosal epithelial wall of the Bowman's capsule. Furthermore, in *Bdellostoma*, the writer never found a tubule entering the central mass. The tubules enter the central duct and the latter alone enters the central mass, hence in *Bdellostoma* Semon's 'Innentrichter' of the pronephric tubules really open into the central duct. Therefore, the inner ends of the pronephric tubules cannot correspond to that end of the tubule of a mesonephric Malpighian body which opens into the Bowman's capsule, and hence the central mass of the pronephros cannot correspond to the glomerulus of a Malpighian body of the mesonephros.

In every pronephros of *Bdellostoma* examined by the writer the central mass has one or more openings, through which the central duct communicates with the lumen of the pronephric vein (figs. 12, 20, 21, 22). These openings are natural, and the endothelial sheath which surrounds the central mass lines the sides of the openings. In the lumen of the duct near these openings, or even projecting into them, there is almost always a rounded collection of nuclei and granules, which is more deeply stained than the central mass. The nuclei and granules do not resemble blood corpuscles, but have the appearance of waste particles. These openings do not occur in the central duct at

any place where the latter is not surrounded by the central mass, nor do they occur in all those segments of the duct which are ensheathed by the central mass. There is no regularity in the location of these openings, except that they are always confined to the posterior half of the pronephros. As is shown by figures 20, 21 and 22, by means of these openings the lumen of the pronephric vein is in actual communication with the pericardial cavity through the central duct and the tubules. Near the openings blood corpuscles are numerous in the lumen of the central duct, and they extend far up the tubules toward the openings of the latter into the pericardial cavity. In one specimen blood plasma extends from the central duct two-thirds of the entire length of four or five tubules. The pericardial cavity communicates with the peritoneal cavity through the large pericardo-peritoneal foramen, and the peritoneal cavity opens into the cloaca through the genital pore, hence we have in Myxinoids the strange condition of the vascular system being open to the exterior of the body. The writer found no traces of blood in the pericardial cavity. Since there are many hundreds of tubules in each adult pronephros, it is difficult to explain why blood is not poured into the pericardial cavity. Price ('10) found that carmine grains injected into the peritoneal cavity through the genital pore of living animals were present later in abundance in the blood taken from different parts of the body. He presumed that the carmine grains gained admission to the blood stream through the ciliated pronephric tubules, that is to say any current in the tubules which is caused by ciliary action is from the exterior toward the interior. This inference is corroborated by the fact that, in almost all the tubules examined by the writer, the free ends of the granular processes extending from the epithelial cells of the tubules are directed inward toward the central duct.

In his description of the development of the pronephros Price ('04, p. 137) states that the manner in which the central duct becomes shortened as the tubules are crowded together is a point which has not been worked out; bending of the duct will not account for all of it. Kirkaldy ('94, p. 356) thinks the duct breaks down and becomes the central mass. Morphological evidence



supports this hypothesis somewhat. The diffused condition of the cells of the duct where the latter is in contact with the central mass suggests that the duct is breaking down, and the enlarged nuclei in the mass, which are without granules, are suggestive of disintegrating cells, while the rounded masses of small nuclei and granules found in the lumen of the duct may be interpreted as nuclei which have been broken down. These may either be ejected into the body cavity through the tubules and pericardial cavity, or, what is more probable, they may be swept into the blood stream and be engulfed by leucocytes. These masses occur only in or near the openings in the central mass which connect the central duct with the pronephric vein. In one specimen, however, a small ball of these nuclei was observed in a tubule. Furthermore, in those pronephroi in which the central duct is most continuous there is no central mass in the anterior part of the duct where its columnar walls remain entire, but the mass is limited to the posterior region where the walls of the duct have lost their columnar structure and the lumen of the duct is surrounded by the mass of nuclei. Also in those specimens in which the duct is broken up into segments and the palisade wall has broken down, each segment, anterior as well as posterior, is surrounded by the central mass, but even in these specimens the bulk of the central mass is found at the posterior end of the pronephros, where the crowding of the original tubules is the greatest. On the other hand, the quantity of the central mass in the older animals seems greater than would be the case if it were all derived from merely a crowding-together of the central duct, especially if it were disintegrating all the while.

The central mass is the same in appearance and structure wherever it occurs in *Bdellostoma stouti*. No lymphatic tissue such as Weldon ('84) describes is present at the posterior end of the pronephros of *Bdellostoma stouti*, unless he refers to the central mass around the duct.

The pronephros of *Myxine* differs strikingly from that of *Bdellostoma* in the fact that it contains much more connective tissue. The pericardial sheath around the tubules is farther from the wall of the tubule than in *Bdellostoma*, and the intervening



space is usually filled with connective tissue. Also, there is much more connective tissue at the base of the pronephros, separating the latter from the pronephric vein, than in *Bdellostoma*, and bands of connective tissue even extend into the vein and into the central mass. In the older animals there is often considerable connective tissue between the walls of the tubule and the surrounding sheath of pericardium (figs. 23 and 24). The pericardial sheath is heavier and its nuclei larger than in *Bdellostoma*. Figure 25, a cross section of one lobe of a pronephros of an adult female, shows the proportion of connective tissue with reference to the size of the tubules which it surrounds. Not all of the tubules in the pronephroi of this animal, however, have connective tissue between their walls and the pericardial sheath. In the youngest specimen examined, a young female 31 cm. long (no. 18), there is very little, and often no connective tissue at all, near the distal ends of the tubules. In the oldest specimen (*Myxine* no. 10) the connective tissue around the tubules and in the entire pronephros is more abundant than in any of the other animals and contains many blood vessels. In this specimen there is, in transverse section of almost every large tubule, a single large vessel which partially or entirely surrounds it (fig. 26). A blood sinus, which completely fills the space between the tubules, as is frequent in *Bdellostoma*, is rare in *Myxine*. The blood around the tubules is almost always confined in vessels which lie in the connective tissue surrounding the tubules.

Unlike *Bdellostoma*, the tubules in an adult *Myxine* are not all of the same size (fig. 27). They are practically of the same size in the youngest specimen examined (*Myxine* no. 18), but in the older animals there is a gradually increasing number of tubules which are slightly larger than others, and in the oldest specimen (*Myxine* no. 10) there are present, in the pronephros, tubules which range in size from the smallest to very large ones. In *Myxine* no. 18 the average dimensions of the tubules are: Width of lumen 0.0162 mm., width of each wall 0.0189, outside diameter 0.054 mm. Only a few of this size are present in the pronephros of *Myxine* no. 10, many approximating the following dimensions: Width of lumen 0.108 mm., width of each wall 0.027, out-

side diameter 0.1620 mm. One tubule in *Myxine* no. 10 is very large, the outside diameter being 0.675 by 1.1475 mm. before it breaks up into smaller branches. The smaller tubules have practically no connective tissue between the walls and the pericardial sheath, the medium size tubules a little, while the large ones usually have a comparatively large quantity of connective tissue around them. The smaller tubules have the appearance of being recent branches from the larger ones.

Kirkaldy ('94) states that, in a *Myxine* with large eggs, the tubules at the posterior end of the pronephros are entirely different from those of a *Myxine* without eggs in that the former are considerably degenerated. The writer is of the opinion that this degeneration of the tubules is not so much a matter of being with or without eggs as one of age. Kirkaldy does not state whether the animal without eggs was young or old. In all the pronephroi of *Myxine* examined by the writer the inner ends of almost all the tubules have disintegrated, only occasionally is one found whose walls remain intact to its opening at the inner end. Only in the oldest specimen (*Myxine* no. 10), which does not have any eggs, are there tubules which have the appearance of degeneration. Some of the largest tubules alone are degenerating, and answer to the description given by Kirkaldy. The nuclei are enlarged, here and there, in the walls, while some of them are attenuated and almost fibrous, and connective tissue is replacing the palisade cells. These degenerating tubules are not numerous and are not limited to the posterior end of the pronephros. They are surrounded by many smaller tubules which give no evidence whatever of degeneration, but on the other hand look like recently formed branches. The cells of the latter are more uniform in size and shape and stain more deeply than those of the large tubules.

One of the most striking differences between the pronephroi of *Bdellestoma* and *Myxine* is that in the latter the disintegrating process of the interior has proceeded further than in the former. In *Bdellestoma* the inner ends of the tubules are always entire, while in *Myxine* they are seldom so. In places only dense lines of nuclei indicate where the inner ends of tubules have been, and

these lines are always streaming toward a more or less large, loosely arranged mass of nuclei, bordering or lying in the pronephric vein.

Investigators do not agree as to the existence of a central duct in the pronephros of *Myxine*. W. Müller and Kirkaldy affirm, while Semon, Spengel and Maas deny the presence of the duct. After studying the pronephros of *Bdellostoma*, it is inevitable for one to conclude that a central duct at one time existed and that fragments of it still exist in *Myxine*. It is not at all prominent and might easily be overlooked except in a comparative study. Along the entire extent of the pronephros the inner ends of the tubules, as stated above, end as a loosely arranged mass of nuclei in which a cavity frequently appears (fig. 28). This cavity is the remains of the lumen of the central duct. In occasional places it opens into the lumen of the pronephric vein, and blood corpuscles occur in it and in the tubules near it (fig. 29). Here and there the central duct is cut in longitudinal section and is then unmistakable (fig. 30). The youngest specimen (*Myxine* no. 18) shows the duct but imperfectly (fig. 31). Likewise, in the oldest animal examined (no. 10) the duct has almost entirely disappeared, the lumen being visible for only a short distance in the posterior part of the pronephros and having but a small piece of columnar wall (fig. 32). Extending along the wall of the vein next to the pronephros is a large number of heavily stained nuclei, which are interpreted as remains of the central mass; or, in other words, remains of the central duct and the inner ends of the tubules. Waste particles, nuclei, and granules are occasionally seen in the lumen of the duct and in the tubules. The tubules are filled with a coagulum which is more or less drawn out into granular processes, and these are almost always directed inwards. In only a few places do the walls of the duct still remain entire (fig. 33). Where the duct has entirely disappeared, the tubules often border directly on the vein and their inner ends are degenerated.

The central mass in the pronephros of *Myxine* is decidedly different from that in *Bdellostoma*. In the young, immature animals (*Myxine* no. 18) it approaches in structure and appearance

the condition existing in *Bdellostoma* (fig. 34). Here the mass contains the more or less oval nuclei, no blood corpuscles, but the cytoplasmic groundwork is replaced by connective tissue. Also, one side of the mass projects into the lumen of the pronephric vein and has a more or less rounded form, while the other side remains attached to the base of the pronephros, but is not as compact as in *Bdellostoma*. The mass is distributed along the entire length of the vein, from the anterior to the posterior end of the pronephros, and extends behind the posterior end of the latter about 0.18 mm. in one specimen. This posterior extension of the mass is cylindrical, has a tubule which ends blindly in it, and contains several large blood corpuscles in capillaries.

In the older animals the central mass is much more unlike that of *Bdellostoma*. In the anterior part of the pronephros it consists of a loose collection of nuclei which extends along the base of the tubules, embracing their inner ends, either lying outside the vein or projecting slightly into it. The mass is not at all compact, and has no definite form. Many of the nuclei are distributed in an irregular manner along the sides of the vein. At the base of the pronephros the mass is more or less exposed to the blood in the vein, even though it does not project into the lumen of the latter. In occasional places the lumen of the vein opens through the mass (fig. 32) and communicates with the lumen of the central duct, and here blood corpuscles are present in the duct, but are rarely found in the tubules. In the posterior part of the pronephros the central mass becomes cylindrical and compact, and projects into and extends posteriorly in the lumen of the vein, completely surrounded by blood. It is bound to the sides of the vein by broad bands of connective tissue which extends from the side of the mass here and there (fig. 35). Blood vessels run from the connective tissue at the base of the pronephros into these bands of connective tissue. The fibers of the latter continue to the center of the cylindrical mass, and enlarged nuclei and blood capillaries are arranged in a cellular structure around this central core of fibers. The giant blood corpuscles are rounded in shape and are contained in capillaries. They are very numerous in the central mass in *Myxine* (as many as fifteen in one sec-



tion taken at random) and average 0.0081 mm. in diameter. Besides the giant corpuscles, there are many large nuclei in the central mass which are stained about the same as the nuclei of the tubules and which contain very many small granules. The giant blood corpuscles resemble those in *Bdellostoma*, but, in the latter animal, they are not nearly as numerous as in *Myxine*. In this posterior region of the pronephros of *Myxine* the central mass resembles a lymphatic structure.

Another striking difference between the pronephros of *Myxine* and that of *Bdellostoma* is the presence of broad bands of connective tissue which extend across the lumen of the pronephric vein from side to side, and partially or completely divide the lumen longitudinally in two parts (fig. 36). These bands are present in all the pronephroi of *Myxine*, from the youngest to the oldest, and are not confined to any particular region of the pronephros. In one place, near the Malpighian body of the pronephros, a large, isolated, thick-walled duct begins blindly in such a band, continues in it for 16 sections and ends blindly. This duct has, on one side, the columnar cells characteristic of the central duct, on the other the appearance of a Malpighian body. From its posterior end a small tubule is given off which extends alongside the duct for five sections then ends blindly. The entire structure measures 0.24 mm. long by 0.195 mm. wide. In another *Myxine* a similar duct arises in one of these bands of connective tissue, extends posteriorly in it for 24 sections (0.36 mm.) and ends blindly at the side of a structure represented in figure 38, without connecting with a tubule which extends from the posterior end of the latter. These structures in the bands of connective tissue are interpreted as degenerating Malpighian bodies. Bowman's capsule is still present, and the tubules are connected with this, but the glomerulus is almost entirely gone.

*Malpighian body of the pronephros.* At least one Malpighian body is always found lying beside the posterior end of the pronephros of *Bdellostoma* and *Myxine*. Investigators of the head-kidney of *Myxinoids* differ as to what part of the excretory system this Malpighian body belongs. W. Müller's figure 2 shows two of them in the pronephros. He failed to state directly



whether he thought they belong to the pronephros or to the mesonephros. Kirkaldy seems to consider the Malpighian body as belonging to the head-kidney. Semon ('96) thinks it is the first Malpighian body of the mesonephros. Spengel ('97) is of the opinion that this is, in fact, the glomus of the pronephros in *Myxine*. From his investigation of very young *Myxine* Maas ('97) concludes that it is the glomus of the pronephros formed by the 'concentriren' of the most posterior 'Gefässnetze' which surround the original segmental pronephric tubules. Price ('04, '10) regards this Malpighian body in *Bdellostoma* as belonging to the 'pronephros,' and from embryological study concludes that it arises by the fusion of glomeruli which are formed in connection with some of the most posterior original tubules of the pronephros before they are crowded together by the posterior movement of the gills. Since, in the embryo, only two or three of the original tubules which take part in the formation of the head-kidney have glomeruli, then the definitive pronephros represents the fusion of not more than two or three glomeruli.

The adult condition of the Malpighian body in *Myxine* and *Bdellostoma* supports the conclusions of Maas and Price in regard to the manner in which it is formed. The pronephros of both shows evidence of being the result of fusion of two or three glomeruli. The following description is for both *Bdellostoma* and *Myxine*, and any variations which either shows will be noted.

The Malpighian body is always located beside the posterior half of the pronephros, usually at the extreme posterior end. It is always imbedded in the connective tissue beside the pronephric vein and is separated from the pronephros by the sheath of its Bowman's capsule and the pericardium, as well as by a more or less wide strip of the pericardial cavity (fig. 39). Quite frequently the Malpighian body is followed immediately by a second, the anterior end of which may lie beside the last few tubules of the pronephros (fig. 12). Ordinarily, however, this second Malpighian body is located back of the posterior limits of the pronephros. The capsules of the two Malpighian bodies shown in figure 12 are connected by a very narrow duct, the short columnar cells of which do not have as much cytoplasm as those of the

pronephric tubules and they absorb much stain. The second Malpighian body is interpreted as being the first Malpighian body of the mesonephros. It is connected by a tubule to a short piece of the segmental mesonephric duct which lies in the lumen of the pronephric vein. The vein ends blindly with this piece of the mesonephric duct a few sections posterior to the Malpighian body. Furthermore, two pronephric tubules, which open into the capsule of the Malpighian body of the pronephros, extend into the pronephric vein, unite and enter the piece of mesonephric duct. Surrounding the base of these tubules, just before they enter the mesonephric duct, is a small amount of the central mass. This short piece of duct contains waste particles throughout its entire extent. The continuous duct of the mesonephros, in this specimen, begins 5 mm. posterior to this short piece and there is no connection whatever between them. When there is but one Malpighian body it usually ends blindly, although the capsule may be drawn out posteriorly into the very small duct which ends in the connective tissue.

In shape the Malpighian body of the pronephros of both *Bdellostoma* and *Myxine* is usually an elongated oval, although it may be almost round or flattened somewhat laterally. In *Myxine* one end is, as a rule, more pointed than the other.

The glomerulus of the Malpighian body in *Bdellostoma* is comparatively compact and uniform in structure. The surface is smooth but slightly convoluted, and is covered by the epithelial lining of Bowman's capsule. There are lines of division which separate the glomerulus here and there into lobes (fig. 40), and in almost every specimen are one or more small cavities, inside of which are blood corpuscles or blood plasma.

In appearance the glomerulus of the youngest *Myxine* (no. 18) is very similar to that of *Bdellostoma*. In the older specimens, however, the lobed condition is more apparent, it is much less compact and has a more or less shriveled appearance, and the spaces in the glomerular mass are more numerous and larger. Webs and strands of connective tissue appear everywhere in the glomerulus and around the outside of the lining of Bowman's capsule. In the oldest *Myxine* (no. 10) these changes are most

advanced (fig. 41). As this figure shows, one large blood sinus has appeared in the glomerulus. This sinus contains corpuscles and blood plasma, and extends almost the entire length of the glomerulus. It has an epithelial lining, outside of which are several concentric layers of connective tissue. On the opposite side of the glomerulus there is a large space with comparatively few nuclei, but entirely filled with slender fibers of connective tissue. This space also extends almost the entire length of the glomerulus. The lining of Bowman's capsule is an epithelium surrounded by a broad band of concentric layers of connective tissue.

In one *Bdellostoma* and one young *Myxine* the glomerulus of the pronephros is double; two distinct glomerular masses are contained in the same Bowman's capsule. In the *Bdellostoma* the capsule is constricted to one-half its width at the point of union between the two glomerular masses, while in the young *Myxine* there is but a slight constriction in one side of the capsule (fig. 42).

In all the pronephroi of both animals, one or more pronephric tubules open into the cavity of Bowman's capsule of the Malpighian body. The capsule in every specimen of *Bdellostoma* is connected with the pericardial cavity by a more or less long, very narrow duct (approximately one-half as wide as a pronephric tubule). In addition to this duct there may be one or more very small openings through the wall of the capsule which connect the cavity of the latter with the pericardial cavity. The capsule of the specimen represented by figure 12 has seven such openings. In none of the specimens of *Bdellostoma* are these openings into the pericardial cavity greater than two or three one-hundredths of a millimeter in diameter.

Spengel ('97) considers the glomerulus of the pronephros to be really a glomus because it hangs freely in a cavity which he found communicated with the pericardial cavity through a very large aperture; in one specimen the opening extended through nineteen sections each 30 microns thick, or 0.57 mm. Semon ('97) never saw in his preparations a communication as wide as Spengel described. Maas ('97) also considers the glomerulus a

true glomus. In each of his youngest *Myxine* he found a very large opening connecting the capsule with the pericardial cavity; it extended through almost as many sections as the glomus itself. But in advanced stages, by a folding of the epithelial wall, a capsule is formed around the glomus, and only a slit-like communication with the pericardial cavity is left, and even this may be completely closed. The glomerulus of the youngest *Myxine* examined by the writer (no. 18) lies directly exposed to the pericardial cavity through an opening 0.156 by 0.192 mm. and resembles a glomus (fig. 43). The opening in *Myxine* no. 15, an adult, measures 0.0195 by 0.105 mm., and that of *Myxine* no. 10, the oldest specimen, is 0.195 by 0.120 mm.

The glomerulus of the head-kidney is not always as large in comparison with the glomeruli of the mesonephros as would be expected if it were formed by the fusion of two or three glomeruli, as the following measurements will indicate; see opposite page.

The figures 1, 2, 3, 4 in the column under 'Glomerulus' represent the number of the glomerulus, counting that of the head-kidney as 1; glomeruli 2, 3, and 4 are the first, second and third glomeruli, respectively, of the mesonephros immediately following the glomerulus of the pronephros. All the glomeruli which lie posterior to the glomerulus of the pronephros are considered as belonging to the mesonephros. The glomeruli of the latter, posterior to the most anterior three or four gradually diminish in size. None of the anterior glomeruli of the mesonephros of *Myxine* were sectioned, therefore no measurements of these are given in the above table. As the table shows, the glomerulus of the pronephros does not always exceed or even equal in size some of the most anterior glomeruli of the mesonephros.

As has already been noted, the Malpighian body of the pronephros of one *Bdellostoma* (fig. 12) is connected by tubules to a fragment of the segmental duct of the mesonephros, which is entirely disconnected from the continuous part of the duct. No other case of a communication between the Malpighian body of the pronephros and the duct of the mesonephros was found in either *Bdellostoma* or *Myxine*. In a young male *Bdellostoma* (no. 22), however, the mesonephric duct on each side continues

| SPECIMEN                | SIDE  | GLOMERULUS | WIDTH      | LENGTH     |
|-------------------------|-------|------------|------------|------------|
|                         |       |            | <i>mm.</i> | <i>mm.</i> |
| Bdellostoma no. 4.....  | Right | { 1        | 0.46       | 0.78       |
|                         |       | { 2        | 0.34       | 0.75       |
|                         |       | { 3        | 0.60       | 1.04       |
|                         |       | { 4        | 0.48       | 0.92       |
|                         | Left  | { 1        | 0.50       | 0.95       |
|                         |       | { 2        | 0.39       | 0.65       |
|                         |       | { 3        | 0.49       | 1.03       |
|                         |       | { 4        | 0.47       | 0.785      |
| Bdellostoma no. 6.....  | Right | { 1        | 0.39       | 0.66       |
|                         |       | { 2        | 0.4485     | 0.60       |
|                         |       | { 3        | 0.468      | 0.66       |
|                         | Left  | { 1        | 0.390      | 0.675      |
|                         |       | { 2        | 0.4485     | 0.705      |
|                         |       | { 3        | 0.30       | 0.4875     |
| Bdellostoma no. 10..... | Right | { 1        | 0.41       | 0.60       |
|                         |       | { 2        | 0.27       | 0.2925     |
|                         |       | { 3        | 0.2925     | 0.405      |
|                         |       | { 4        | 0.41       | 0.60       |
| Bdellostoma no. 15..... | Right | { 1        | 0.39       | 1.10       |
|                         |       | { 2        | 0.40       | 0.30       |
|                         |       | { 3        | 0.40       | 0.50       |
|                         |       | { 4        | 0.65       | 0.65       |
|                         | Left  | { 1        | 0.4485     | 0.88       |
|                         |       | { 2        | 0.39       | 0.40       |
|                         |       | { 3        | 0.40       | 0.60       |
|                         |       | { 4        | 0.53       | 0.84       |
| Bdellostoma no. 16..... | Left  | { 1        | 0.41       | 0.60       |
|                         |       | { 2        | 0.2925     | 0.45       |
|                         |       | { 3        | 0.2925     | 0.45       |
|                         |       | { 4        | 0.39       | 0.51       |
| Myxine no. 10.....      | Right | 1          | 1.2675     | 1.305      |
| Myxine no. 11.....      | Left  | 1          | 0.8775     | 0.975      |
| Myxine no. 15.....      | Left  | 1          | 0.6825     | 0.84       |
| Myxine no. 18.....      | Left  | 1          | 0.4095     | 0.348      |



to the posterior end of the pronephros, as is shown in figures 1 and 2. When the anterior ends or the ducts of this animal were sectioned, it was found that the lumen of the right duct ends beside the posterior end of the Bowman's capsule of the Malpighian body of the pronephros, but there is no communication between them. Some of the sections of the left duct were scraped off the slide in this region, hence the duct could not be followed. Immediately posterior to the pronephros the lumen of the right duct of this animal abruptly enlarges, becoming a cavity which measures 0.4875 by 0.6240 by 1.335 mm. This cavity is almost entirely filled with a rounded, loose mass of nuclei which resembles a disintegrating glomerulus. This mass is connected here and there to the lining of the duct by narrow strands of nuclei, but no blood vessels could be seen in them. The lining of the duct here does not resemble that of a Bowman's capsule, and is composed of short columnar cells like those of the pronephric tubules. If this be a disintegrating glomerulus, it is difficult to explain why it should be in the lumen of the mesonephric duct. Immediately posterior to this enlarged cavity the duct becomes narrowed to a diameter of 0.117 mm.

### *Mesonephros*

*Bdellostoma*. *Mesonephric ducts*. Except in specimen 22, the mesonephric ducts of the *Bdellostomae* examined begin from 2 to 10 mm. back of the posterior end of the pronephros. Small strands of tissue, resembling in structure the outer wall of the duct, extend from the anterior end of the duct toward the pronephros. Sometimes a lumen is present in one of these strands for a varying, but short, distance. These strands may be the only trace of pro- or mesonephric elements between the posterior limit of the pronephros and the anterior end of the ducts, or, in addition to these, there may be one or more isolated tubules or traces of glomeruli in this intermediate region.

The anterior ends of the right and left ducts in an individual are seldom opposite each other. The duct does not start abruptly, but is always narrowest at the anterior end, and presents the

appearance of having at one time extended farther forward. There is no uniformity in the diameter of the duct at its anterior end, except that it is always smallest here, varying in width from a mere thread to 1 mm.

The left duct usually bends abruptly laterad at its anterior end, until it assumes a position close beside the left postcardinal vein, and retains this position to the end of the body cavity. The right ducts bends only slightly laterad at its anterior end to assume a similar position beside the right postcardinal vein. The right duct is invariably closer to the dorsal aorta than the left.

In adults of the same size, the ducts of the males are larger and longer than those of the females. There is not much variation in the size of the two ducts of a given animal, nor in the ducts of all the adult animals of the same sex. Each duct is smallest at its anterior end, and gradually increases to its largest size at the posterior end of the body cavity, where it is from 1.5 to 2 mm. wide in females and from 3.5 to 4 mm. in males.

Each duct is flattened dorso-ventrally throughout its entire length, being most flattened at the posterior end of the body cavity. The outer surface of the ducts is smooth. When a duct is stained, cleared and examined under the microscope the surface appears longitudinally striated, due to the occurrence of ridges formed in the lumen of the duct by the columnar epithelial lining.

The ducts lie immediately ventral to the notochord, separated from the latter by a small cavity which extends the entire length of the ducts. By cutting the peritoneum along the dorsal body wall at the sides of each duct, the ducts, together with the postcardinals and the dorsal aorta, can easily be removed.

The mesonephric ducts of the adult male are unlike those of the adult female in some respects. Those of the former are not only larger, but are longer, by reason of lateral bendings or convolutions. Figure 45, a dorsal view, represents the typical appearance of the male ducts, and figure 46 those of the female. At their anterior ends the ducts of the male are only slightly convoluted, but, beginning about ten somites posterior to the ante-

rior ends, the convolutions become quite pronounced and regular. There is but one convolution in each somite; the ducts bend laterad at the interseptal lines and mediad between these lines. The convolutions continue to the posterior end of the body cavity, but in the last five or six somites they diminish in extent. The greater size of the male duct is caused principally by the fact that its lumen is larger than that of the female. The walls of the male duct, however, are somewhat coarser than those of the female.

No such convolutions occur in the ducts of the females. The ducts bend laterad more or less slightly at the interseptal lines, but there is no suggestion of the convoluted condition of the male ducts.

At their posterior ends, the ducts of both males and females have the same gross structure and appearance. The two ducts leave the peritoneal cavity at its posterior end, approach each other and continue a short distance (5 to 10 mm.) along the dorsal surface of the genital chamber, then converge sharply. At the point of convergence the ducts bend ventrad and laterad, and become abruptly narrowed to very small tubes (figs. 47 and 48). The latter continue posteriorly, bend sharply ventrad at the posterior end of the cloaca, and open in this region of the cloaca on a papillary enlargement on a prominent ridge which extends along the dorsal wall of the genital chamber and cloaca. The two openings lie alongside, but one is usually slightly posterior to the other.

The ducts of young males (fig. 49) are not convoluted at any place, and resemble in appearance and gross structure those of adult females.

*Histology.* Figures 50 and 51 are camera lucida drawings of transverse sections of a male and female duct, respectively, taken from the mid-region of the body cavity. The section of the male duct was selected where there is the smallest possible effect on the internal structure because of convolution. As the figures show, the ducts consist of an inner epithelium and an outer envelope of connective tissue.

The epithelium is composed of columnar cells, and is arranged in a series of longitudinal folds or ridges. Wax models show that these ridges branch and anastomose freely, but are always longitudinal and the branching is dichotomous. The two branches may run parallel to each other for a short distance and then unite, or may unite with another ridge, or may end without union to other ridges. A ridge may be only a fraction of a millimeter long and be entirely unattached to any other ridges. When free, the ends of the ridges rise gradually from the normal height of the epithelium.

The ridges are present in all parts of the duct, from the anterior to the posterior end, but at the former end, where the duct is small, they may be only two or three in number (fig. 52). As the duct becomes larger, the ridges increase in number, and are most numerous at the posterior end where the duct reaches its maximum width. The ridges vary in number in a transverse section in corresponding regions in different adult specimens, as is indicated by the following table:

| SPECIMEN                        | OUTSIDE DIAMETER<br>OF DUCT | NUMBER OF<br>RIDGES | HIGHEST<br>RIDGES |
|---------------------------------|-----------------------------|---------------------|-------------------|
| Bdellostoma no. 15, female..... | 0.546 x 1.0725              | 20                  | 0.1365            |
| Bdellostoma no. 17, female..... | 0.351 x 1.365               | 29                  | 0.0780            |
| Bdellostoma no. 16, male.....   | 0.780 x 2.4375              | 26                  | 0.390             |

There is considerable variation in the height of the ridges in the same transverse section and in different parts of the same duct. Figures 50 and 51 show the variation in the same section. For a given duct the ridges are lower at the ends than in the mid-region.

The columnar epithelial cells which form the walls of the duct are shortest between the ridges. The nucleus is always located about one-third the length of the cell from the basal membrane. The cytoplasm is very granular throughout the entire cell body, the granules being especially numerous and large at the distal ends of the cells. Along the surface of the distal ends of the cells are agglutinations of granules which project into the lumen of the duct and resemble the mass of waste material which is

seen in the center of the lumen of the duct throughout its entire length. Toward the distal ends of the cells are numerous small and large, yellowish, round or oval bodies of homogeneous structure, which resemble oil droplets. Some of these are minute granules, while others are as large as the nucleus of the cell. They are especially numerous and large in the cells on top of the ridges, and are distributed in no definite manner. Only a few are found in the cells between ridges. These bodies are probably what W. Müller described as yellowish pigment granules. The larger bodies do not resemble pigment granules, however.

The long, columnar cells are always arranged on the ridges in the shape of a fan, as illustrated in figure 53. To form a ridge, the entire layer of epithelial cells bends into the lumen of the duct, and the connective tissue outside the basal membrane of the epithelium makes a core which fills the concavity beneath each ridge (fig. 53).

The ridges are more numerous in the adult male ducts than in the adult female. They are also higher, but the cells are no longer than those of the female. The greater height is due to the fact that the epithelial layer extends farther into the lumen of the duct in the males. Wide ridges are more numerous in the male than in the female, some of them being flat on top. The concavities formed by the infoldings of the epithelial layers are larger in the male than in the female, and are also filled by a core of connective tissue.

Maas ('97) has suggested that the mesonephric ducts are not simply excretory, but they may have a secretory function as well. The distribution of the blood vessels, the presence of cilia, the folded epithelium, the enlarged condition of the ducts in comparison to the small tubules of the Malpighian bodies of the mesonephros have been mentioned in the literature as indicative of such a possibility. The many large foldings or ridges of epithelium certainly increase the epithelial surface far beyond what would seem necessary for excretion alone. The enlarged, convoluted condition of the ducts in the males is very peculiar. The presence of the small and very large, yellowish bodies near



the distal ends of the epithelial cells, especially in the large cells of the ridges, is suggestive of secretion.

The envelope of connective tissue surrounding the duct consists of two parts, a loose web containing blood vessels and a more or less compact stratified layer. The loose web lies next to the basal membrane of the epithelium. It follows closely the outline of this membrane, and fills the concavities under the ridges, as shown in figure 53. Some of the blood vessels in this connective tissue contain giant blood corpuscles, like those found in the central mass.

The compact stratified layer consists of fibers arranged in concentric rings around the duct, and is usually thicker along the median surface of the duct. The peritoneum lies next to the stratified layer.

The writer was unable to identify in *Bdellostoma* the three layers which W. Müller describes as forming the connective tissue envelope around the duct of Myxine. Nowhere is there a layer that can be called the 'membrana propria.' The two layers observed by the writer correspond to the 'adventitia' and the layer which contains blood vessels, as designated by Müller.

*Malpighian bodies.* Price has shown that at one time in the development of *Bdellostoma* the mesonephric duct is continuous with the central duct of the pronephros, but that, later, a short piece of the duct posterior to the pronephros degenerates, together with its Malpighian bodies. The amount which degenerates is not always the same, hence in the adult there is considerable variety in the structure of the anterior ends of the ducts and in the location of the first few Malpighian bodies of the mesonephros. The most anterior Malpighian body of the Mesonephros may be located immediately posterior to the Malpighian body of the headkidney and may be attached to the latter and to a short, isolated piece of the mesonephric duct (fig. 12); or it may be completely isolated in the space between pro- and mesonephros. Not only one, but two or three of the mesonephric Malpighian bodies may be in the intermediate space, entirely disconnected from each other and from the mesonephric

duct. Again, the first Malpighian body of the mesonephros may be located at the anterior end of the duct, and its tubule may be widened to form the beginning of the duct, or the latter may extend slightly anterior to the Malpighian body, in which case the tubule enters the duct at the side. Two or three Malpighian bodies may be crowded close together around the anterior end of the duct, but their tubules always enter the lumen of the duct separately and in consecutive order (fig. 54, a drawing of the anterior ends of the ducts of *Bdellostoma* 4). On the left side Malpighian bodies 1, 2, and 3 are very close together. The first two are in one somite, 3 occupying the next segment. When the Malpighian bodies are thus crowded together at the anterior end of the duct, the arteries which supply them arise from the aorta at successive points close to the posterior end of the pronephros, a condition which suggests that these Malpighian bodies have been pushed back from a more anterior location. For example, the point of origin of the artery which supplies the first Malpighian body of the right duct of *Bdellostoma* no. 15 is 3.5 mm. anterior to the Malpighian body, and the latter is only 5 mm. posterior to the caudal end of the pronephros.

Except where the Malpighian bodies are occasionally crowded together at the anterior end of the duct, there is but one in each somite to somite 60-65. The ducts usually begin in somite 33-35, and the body cavity extends to somite 75-79, hence the posterior third of the ducts have no Malpighian bodies. Price learned that in embryos excretory tubules are present in the most posterior somites, but they later degenerate in the last 19 or 20 segments. The most posterior Malpighian bodies in the adult show signs of degeneration. They are small, often not visible to the unaided eye, frequently in a shriveled condition, and parts of them may be lacking; for example, in one case the tubule only is present. This degeneration is of unequal extent in different animals and on both sides of the same animal.

Except an occasional one at the anterior end, all the Malpighian bodies lie along the median side of the duct. There is no regularity in the orientation of the bodies with reference to the somites; they may be located at either anterior or posterior side

or near the middle of the somite. Those of a side are not separated from each other by equal intervals, but, as a rule, are arranged in pairs, a greater distance occurring between two pairs than between the two bodies of a pair (figs. 55 and 56). This paired condition is in some way connected with the arterial supply, as is shown by figure 56. Each of the Malpighian bodies represented in this figure is in a separate somite. The two arteries which supply the two Malpighian bodies of one of these pairs are usually branches from a somatic artery, as shown by figure 56, but sometimes one of the arteries arises directly from the dorsal aorta, close beside the somatic artery. When a Malpighian body is not one of a pair, its artery comes directly from the dorsal aorta. Seldom are the two Malpighian bodies of corresponding somites on each side of the body opposite each other, as is shown by figure 55.

The Malpighian bodies and their tubules usually extend anteriorly to the point where the tubule empties into the mesonephric duct. When, however, each Malpighian body in two adjacent somites is supplied by a branch from the same somatic artery, the anterior one is doubled back upon its tubule so that the distal end points posteriad toward the origin of the artery which supplies it.

Each Malpighian body has a tubule which enters the mesonephric duct. There are two types of these tubules which differ distinctly and which will be designated as 'plain' and 'compound' tubules. All the tubules of a given animal are of one type only.

The plain tubules are composed of very short, columnar cells which have very little cytoplasm, as shown in figure 57. These cells are all approximately the same size. Their nuclei stain much more deeply than either the nuclei of the connective tissue or those of the mesonephric duct, hence the tubules are very distinctly differentiated from surrounding tissues. At the proximal end the cells of the tubule are directly continuous with those of the mesonephric duct, and at the distal end with the cells of the single layer of squamous epithelium which lines Bowman's capsule. The basal membrane of the mesonephric

duct is continuous with that of the tubule. The tubules vary in length, the shortest averaging 0.07 mm., the longest 0.2 mm. When long, they are more constricted than when short, the outside diameter of the narrowest tubule measured being 0.04 mm. The size of the opening where the tubules enter the duct varies, but approximates 0.12 mm. in diameter. Figure 58 is a diagrammatic reconstruction of a plain tubule.

The compound tubule is strikingly different from the plain, as is shown by figure 59, which is a reconstruction of a Malpighian body with a tubule of this type. It consists of two parts, a neck and a trunk. All of the Malpighian bodies of all the specimens examined, except the one described above, have this type of tubule. The neck is that part of the tubule which joins the Bowman's capsule, and in structure and appearance it is exactly like the plain tubules. It consists of short, columnar cells of approximately equal height and which have very little cytoplasm. The nuclei stain much more deeply than those of surrounding tissues, so that the epithelium of the neck is easily distinguished from that of the trunk. The cells of the neck gradually decrease in height as they approach Bowman's capsule, and are continuous with the cells of the single layer of squamous epithelium which lines the capsule. The neck is variable in width and length within narrow limits in different specimens and in different tubules of the same specimen. An idea of the dimensions may be had from one specimen: Outside diameter at entrance to trunk of tubule, 0.35 mm., and at entrance to capsule, 0.078 mm.; length, 0.4 mm. The neck is sometimes as much as twice this length, however. At the proximal end of the neck the epithelial cells and basal membrane are continuous with the cells and basal membrane, respectively, of the trunk of the tubule. The transition from the cells of the neck to those of the trunk is gradual, as shown in figure 60.

The trunk of the compound tubule is exactly like the mesonephric duct in structure. It consists of an epithelium of high columnar cells which are arranged in ridges, and cells and ridges are of approximately the same height as those of the duct. At the anterior end of the duct this portion of the tubule is often as large in diameter as the duct itself. Farther posteriorly, how-

ever, where the duct is larger, the trunk of the tubules is only from one-fourth to one-third as large as the duct. At the opening of the trunk of the tubule into the duct, the epithelial cells of both are continuous with each other, including their basal membranes (fig. 61). When the trunk of the tubule lies close beside the duct, each has its own envelope of connective tissue, but the two are bound together by a compact band of connective tissue arranged in concentric layers. The trunk of the compound tubules varies somewhat in length, but is not more than a fraction of a millimeter, approximating 0.75 mm. The trunk and neck together are about 1.5 mm. long. In some cases the neck may be longer than the trunk, but it is usually much shorter.

Waste granules resembling those in the duct are present in large quantity in the lumen of both the plain and compound tubules along their entire length. The short columnar cells of the plain tubules and of the necks of the convoluted tubules lack the yellowish bodies which are found in the cells of the duct, but the long columnar cells of the trunk of the compound tubules have more or less of them.

Each glomerulus almost entirely fills its Bowman's capsule. The squamous epithelial lining of the capsule continues around the glomerular mass. The capsule is usually oval in shape. The glomeruli are usually largest at the anterior end of the duct, and diminish in size gradually toward the posterior, the most posterior being the smallest. For instance, the following are measurements of glomeruli from different regions of the duct:

| REGION         | SIDE  | WIDTH      | LENGTH     |
|----------------|-------|------------|------------|
|                |       | <i>mm.</i> | <i>mm.</i> |
| Anterior.....  | Right | 0.640      | 0.680      |
|                | Left  | 0.600      | 0.830      |
| Middle.....    | Right | 0.500      | 0.630      |
|                | Left  | 0.480      | 0.645      |
| Posterior..... | Right | 0.345      | 0.351      |
|                | Left  | 0.331      | 0.405      |



The tubules of the Malpighian bodies, also, are smallest and shortest at the posterior ends of the duct. In one specimen the most posterior trace of a Malpighian body is a compound tubule, the base of which opens into the duct, and the neck ends blindly. The neck is 0.06 mm. long by 0.105 mm. wide, and the trunk is 0.15 mm. long by 0.21 mm. wide.

Each Malpighian body is surrounded by a compact stratified connective tissue band which is arranged in concentric rings and which is closely applied to the epithelium of the capsule. Outside this band of connective tissue there may be a more or less loose web of connective tissue which envelopes the Malpighian body and the mesonephric duct. In one section, which shows the short plain tubule opening into both the duct and the capsule, the broad band of stratified connective tissue surrounding the duct continues around the tubule and the Bowman's capsule. Here the tubule is very short and narrow. Figure 62 shows the relation of these tissues when both glomerulus and the basal portion of the tubule are present in the same section with the duct. From this figure it will be observed that the glomerulus and the distal end of the tubule are not entirely separated from the mesonephric duct, but that they are bound to the latter by either a band or a web of connective tissue which envelopes both duct and Malpighian body.

### *Myxine*

*Mesonephric Ducts.* The mesonephric ducts of *Myxine* are like those of *Bdellostoma* in so many respects that a detailed description of them would be needless repetition, hence only points in which they differ will be noted.

The ducts are longer in *Myxine*, of course, and are a fraction of a millimeter narrower than the female ducts of *Bdellostoma*. The ducts of the latter are gray and opaque, while those of *Myxine* are approximately of the same color as the flesh and are somewhat transparent.

The anterior ends of the ducts are farther removed from the pronephros in *Myxine*, being 10 to 20 mm. posterior to it. No

bendings or convolutions were present in the ducts of any of the specimens. The laterad bending at the interseptal lines is somewhat less than in *Bdellostoma*, which may be due to the fact that the somites are larger in *Myxine*. In the adult *Bdellostoma* the somites are approximately 5 mm. wide, while in *Myxine* they are about 6.5 mm.

The histology of the ducts of *Myxine* is practically the same as those of *Bdellostoma*. The long columnar cells are arranged in longitudinal ridges. In the youngest specimen studied (*Myxine* no. 18) the ridges are not formed in the same manner as in *Bdellostoma*. The basal membrane of the epithelium does not bend inward, toward the lumen of the duct, at the base of the ridges, hence no concavities are formed under the latter. Also, the nuclei of the epithelial cells remain at the same level around the entire circumference of the duct and turn inward only very slightly at the base of the ridges. The ridges are formed solely by the elongation of the epithelial cells. The longest cells are approximately 0.0675 by 0.0081 mm. The majority of the nuclei are round, although many are short ovals. There are approximately fifteen ridges in each duct in the mid-region of the body.

In all the adult *Myxines* the ridges in the ducts are more numerous, shorter and narrower than in *Bdellostoma*. Their shape is slightly different from those of the latter animal, and much more uniform. They are usually narrower in the middle than at the ends, and never have a wide, flat surface such as are frequent in *Bdellostoma*. In one specimen, which has eggs 12 mm. long, the duct is 0.39 by 1.2675 mm., and has 54 ridges, each approximately 0.039 by 0.117 mm. The duct of another animal measures, at one place in the mid-region selected at random, 0.2925 by 0.975 mm., and has 53 ridges, each approximately 0.029 by 0.105 mm. The ridges present a finger-like appearance, as shown in figure 63.

The epithelial ridges in the ducts of adult animals do not have concavities beneath them, but the nuclei at the base of the ridges are arranged in a small heap (fig. 63).

The nuclei of the epithelial cells in the ducts of *Myxine* are much nearer the bases of the cells than in *Bdellostoma*. Most of them are round, but some are oval. All the cells have much cytoplasm, and as a rule they are narrower at the middle than at the ends.

A very striking difference between the epithelial cells of *Myxine* and those of *Bdellostoma* is the quantity and distribution of the yellowish bodies. These are present in every ridge in *Myxine*, more in some ridges than in others of the same section, and more in some sections than in others. They are almost entirely confined to the center of the ridge, forming a narrow core which extends from the nuclei at the base of the cells to the top of the ridge (fig. 64), but not to the very distal ends of the cells. At the top of the ridges these yellowish bodies are in rows, which spread out in the shape of a fan, following the arrangement of the cells. The bodies vary in size from tiny granules to a diameter exceeding the width of a cell, and large and small are intermingled in an irregular manner in all parts of the core.

There is much less connective tissue around the mesonephric ducts and Malpighian bodies in *Myxine* than in *Bdellostoma*. The Malpighian bodies are confined to the anterior two-thirds of the duct, one in each somite. They are approximately the same size as those of *Bdellostoma*, the largest at the anterior end of the ducts and the smallest toward the posterior. Only the compound type of tubules were found in *Myxine*, and these are usually shorter than the compound tubules of the Malpighian bodies in *Bdellostoma*.

## 2. REPRODUCTIVE SYSTEM

### *Myxine*

*Female.* The following is a general description of the female generative apparatus in *Myxine*. It is not strictly applicable to any one specimen, for there is much variation within certain limits. Schreiner ('04) has given a very detailed account of the ovary in the European *Myxine*, and examination shows that

the North-American *Myxine* does not present any striking differences.

The single ovary occurs on the right side, and extends from the region of the gall bladder to the posterior end of the coelomic cavity, and is approximately (within 2 or 3 cm.) one-half the entire length of the animal. It is suspended in the body cavity by a single mesovarium, which is attached along its proximal margin to the mesentery where the latter joins the dorsal surface of the alimentary canal, to the right of the supra-intestinal vein. In young animals the mesovarium presents a perfectly flat surface, but in specimens which have eggs 5 mm. long or more it becomes folded transversely, being most folded in the oldest females. The distal margin of the mesovarium does not take part in this folding, but remains straight, therefore the two margins are much shorter than the rest of the mesovarium, a condition which causes the mesovarium to bulge out laterally.

For about 10 mm. at the anterior and posterior ends, the mesovarium is merely a line along the dorsal surface of the digestive tract, then increases more or less abruptly to its average width. At the posterior end it decreases more or less gradually to a line on the dorsal surface of the alimentary canal, and ends at the genital pore. Even in the youngest females, where the mesovarium is simply a flat sheet, its width varies at different points along its course (fig. 65). In successive older stages these inequalities in width are more and more pronounced, the widest points being where the eggs are attached. In the youngest specimens (no. 18) the mesovarium is 2 mm. wide at the widest points, and in *Myxine* no. 2, which has eggs 22 mm. long, it is as wide as 30 mm. where the large eggs are attached. Between these large eggs the mesovarium is from 10 to 20 mm. wide. The weight of the eggs doubtless stretches the mesovarium, for it is drawn out into an elongated strand where each egg is suspended. Frequently these strands are intertwined and even tied in knots. In specimen no. 20 the mesovarium is 25 to 30 mm. wide and the strands to which the corpora lutea are attached extend only 3 or 4 mm. beyond the general width of the mesovarium. In one of the oldest females the



mesovarium is approximately 15 mm. high along its entire course, except at the ends, and its distal margin is not folded, but is comparatively straight. Another old specimen has a mesovarium which is 20 to 25 mm. wide, and its distal margin is also straight. It is probable that, after losing the eggs, the distal margin of the mesovarium tends to become straight again and the excessive width caused by stretching where the eggs were suspended is taken up by much transverse folding. As stated above, the distal margin of the mesovarium does not take part in this folding.

The eggs seldom occupy more than the distal third of the mesovarium, never extending entirely to the proximal margin. In the adult animals they are distributed throughout the distal half of the mesovarium, even to the outermost margin, but in one of the young specimens (fig. 65) the most distal millimeter of the mesovarium is entirely without eggs. The smallest eggs are always most distal, successively larger stages extending proximally, the largest being most proximal (fig. 66).

The eggs are comparatively evenly distributed along the length of the mesovarium, except that there are few, if any, in the most posterior 20 to 30 mm. Eggs larger than 10 mm. long, when present, usually occur at fairly regular intervals along the mesovarium from within 2 to 5 cm. of its anterior end to 3 to 10 cm. from the posterior end. Often from three to four or five large eggs are found together in a cluster, due to the intertwining of the long strands of mesovarium by which they are suspended.

In each female which has normal eggs there are all gradations of sizes of these from mere dots to those 2 mm. long. When eggs longer than 2 mm. are present there are no intermediate sizes between the 2 mm. eggs and the large ones, and all of the latter are within 1 or 2 mm. of being of equal size. The largest eggs found in any of the specimens measure 7 to 8 by 24 to 25 mm.

In immature females the smallest eggs are crowded close together along the entire distal margin of the mesovarium. In older specimens the smallest eggs are much less numerous; clusters of them are separated by more or less wide intervals. Eggs 2 mm. long average approximately one for every 5 to 10



mm. of mesovarium in young females, and even less in adults. The following table shows the number of larger eggs present in a few individuals:

| NUMBER OF SPECIMEN | LENGTH OF ANIMAL | SIZE OF EGGS        | NUMBER OF EGGS |
|--------------------|------------------|---------------------|----------------|
|                    |                  | mm.                 |                |
| 16                 | 50               | 1.5-2.0 x 6.0-7.0   | 37             |
| 8                  | 56               | 3.0-3.5 x 10.0-11.0 | 35             |
| 6                  | 61               | 3.0-4.0 x 13.0-15.0 | 45             |
| 4                  | 61               | 6.0-7.0 x 18.0-19.0 | 33             |
| 2                  | 58               | 8.0-8.5 x 19.0-20.0 | 27             |
| 14                 | 58.5             | 7.0-8.0 x 21.0-22.0 | 28             |
| 11                 | 55               | 8.0-9.0 x 21.0-22.0 | 26             |
| 3                  | 64               | 7.0-8.0 x 24.0-25.0 | 35             |

None of the eggs are so far advanced as to have hooks at the ends.

In shape, the eggs are round until they reach a diameter of about 1.5 mm., then they begin to elongate, and become oval. Eggs 2 mm. long and 1 to 1.5 mm. wide. The eggs are developed between the two layers of peritoneum which form the mesovarium, as shown in figure 74. When the eggs begin to assume the oval shape, the pointed ends project beyond the place of attachment to the mesovarium, and one end evidently grows more rapidly than the other for the largest eggs are usually attached between one end and the equator, leaving the ends free (fig. 67).

Other structures besides eggs may be present in the mesovarium. Specimen no. 20 has 33 empty egg envelopes, the 'corpora lutea' (fig. 68). The largest eggs in this animal are approximately 1 by 1.5 mm. Specimen no. 2 has, besides the 27 large eggs, two corpora lutea, each of which is the same size as the envelopes around the eggs (8.5 by 20 mm.), and which has been opened along one side (fig. 69). The corpora lutea in *Myxine* no. 20 are much smaller (4 by 6 mm.) and are shrunken into a compact mass. *Myxine* no. 5 has 25 or 30 of these corpora lutea which are still smaller (2 by 3 mm.) than those of specimen no. 20, as shown in figure 70. The largest eggs of specimen no. 5 are 1.5 to 2 by 0.5 to 1 mm. Specimen no. 1 has several even

smaller corpora lutea. The writer is unable to explain what is the final fate of the corpora lutea, unless they form some of the small brown oval bodies which will be described later. It seems improbable that they are completely absorbed. The fact that, as noted above, there are no intermediate stages between eggs about 2 mm. long and the large ones, and that also in animals which have corpora lutea, the eggs present do not exceed 2 mm., is interpreted to mean that as soon as some eggs exceed 2 mm. in length all the other eggs are arrested in development until the larger ones have matured and have been passed from the body, and their corpora lutea are well along in the process of degeneration.

Distributed comparatively uniformly along the entire mesovarium, wherever eggs occur, are numerous brown oval bodies which measure approximately 0.5 by 1 mm. They are somewhat flattened laterally, and are located proximally to the smallest eggs. None of these brown bodies are present in the youngest specimens, but they occur in all adult females, being most numerous in the older ones. In the mesovaria of some of the latter, for example specimen no. 9, there are no eggs, but many of these brown bodies. Since only comparatively a few eggs become larger than 2 mm., many eggs do not attain complete development, but degenerate. The brown bodies are the degenerated eggs and their envelopes. Intermediate stages of degeneration between the brown bodies and the normal eggs occur occasionally. It is possible that some of the brown bodies represent degenerated corpora lutea, though none were found whose structure would indicate this. As shown in figures 71, 72 and 73, representing sections of three stages of these, the mesovarium envelopes the brown bodies in the same manner as it does the small eggs (fig. 74), but the walls of the envelope around the former are much thicker. Also, numerous blood capillaries occur between the envelope and the former membranes of the egg, which have been converted into convoluted strands of connective tissue. The center of the brown bodies is filled with round nuclei and dark-yellowish granules which may be the remains of the yolk and which cause the brown color.

Along the posterior 25 or 30 mm. the mesovarium of young animals has a narrow band (0.5 mm. wide) of testis lobes, as shown in figure 65, but there are no traces of such testis lobes in any of the nine adult females which have normal eggs. A few small eggs are occasionally found in the mesovarium proximal to these testis lobes, but only at the anterior end of the testis band. The posterior end of the mesovarium in adult females with normal eggs may contain a few small eggs or brown bodies, but it is usually without any reproductive elements.

Sections were made of portions of the bands of testis lobes found in two of the young animals, viz., no. 18, no. 7. Figure 75 is a transverse section of the band in *Myxine* no. 18. The structure is similar to that of the young European *Myxine* as described by Nansen ('87), Cunningham ('87 and '92), and Schreiner ('05). The testis band consists of a large mass of stroma cells and many primitive germ cells which lie among the stroma cells. The band is attached to the digestive tract by the continuation of the mesovarium, though here it may perhaps be called more properly the mesorchium. Here and there, in a transverse section of the band, is a small follicle formed by a single layer of stroma cells, inside of which are from four to two or three dozen spermatogonia. No mitotic figures are present in any of the spermatogonia. On the contrary, the cells are in a resting stage, the nucleolus being visible in most of them. The entire mass is surrounded by the squamous epithelium of the mesorchium.

*Myxine* no. 7 is older than no. 18, and its testis lobes are more advanced in development, as is shown by figure 76. The follicles are more numerous, are larger, and contain more spermatogonia. The stroma cells are much less numerous. No mitotic figures are present, but the chromatin of many of the spermatogonia is scattered throughout the cell and the nucleolus has disappeared. The epithelium which surrounds the testicular mass is thicker, and here and there it turns inward, thus cutting the testis band into lobes. Small eggs occur along the entire distal margin of the mesorchium anterior to the testis band. The follicles of neither no. 18 nor no. 7 contain any spermatozoa. These specimens are

not sufficiently advanced in development to indicate whether they will eventually be male or female.

*Male.* Myxine no. 15, an animal 62 cm. long, has a testis band 5 mm. wide along the distal margin of the posterior 9.5 cm. of the mesorchium. Anterior to this band the mesorchium is of comparatively uniform width (about 7 mm.) all the way to the anterior end, and contains along its distal margin numerous brown bodies (degenerated eggs) but no normal eggs. No brown bodies, however, are present in the portion of the mesorchium which is occupied by the testis band. The mesorchium is not folded, but is straight and flat (fig. 82). There are no indications that this animal ever produced large eggs, and it is considered as an almost mature male. Only about one-half of the follicles contain cells which show mitotic figures, but no spermatozoa were found. A transverse section of the testis band (fig. 77) shows that, in this specimen, the large follicles are larger than the largest ones in specimens no. 18 and no. 7, but they range in size from small to large and are closely crowded together, so that the stroma cells are limited to narrow strips between the follicles or between the latter and the mesorchial sheath. Almost every stage of mitosis is represented. Not all the cells of an individual follicle show mitotic figures, but what figures are present are approximately in the same stage (fig. 78).

A striking difference between the testis band of Myxine no. 15 and those of specimens no. 18 and no. 7 is the fact that the single layer of squamous mesorchial epithelium which envelopes the entire band has been here and there converted into a columnar epithelium. The long, spindle-shaped cells of the mesorchial sheath become shortened and arranged in palisade order. These columnar cells have not entirely surrounded the testis band, but in places the squamous epithelium is still present and forms the sheath of the band.

In two other specimens, no. 12 and no. 13, this process of conversion of the mesorchial sheath into columnar epithelium has advanced until the entire testis band is enveloped by it. The band in these two animals is very different from that of any other specimen. In the first place, the band extends the entire



length of the mesorchium (fig. 83). Specimen no. 12 has the general appearance of being older than no. 13. At the anterior end of the genital fold the width of the mesorchium and the testis band together is 4 mm., and it increases at once to 5 mm., then fluctuates between 4 and 5 mm. to a point about 7 cm. from the extreme posterior end. At this point the combined width of the band and the mesorchium increases to 7 mm. and remains this to the posterior end of the mesorchium. The testis band is suspended from the distal end of the mesorchium, and at the anterior end it is 1 mm. wide by 0.5 mm. thick. The band increases gradually in both width and thickness as it proceeds posteriorly, and at mid-region it is 2 mm. wide by 0.75 mm. thick. At a point 7 cm. from the posterior end it reaches its maximum size, 6 mm. wide by 1 mm. thick, and retains these dimensions to the extreme posterior end, immediately in front of the genital pore. The testis band of specimen no. 13 is neither as high nor as thick as that of no. 12, the widest part being 5 mm. Also, it is not as lobulated as the band of no. 12, and it is yellowish in color instead of reddish as in no. 12. In the latter the small lobules give a granular appearance to the band, which is especially pronounced in the posterior region. There is not the slightest trace of eggs, normal or degenerated, at any place in the mesorchium of either no. 12 or no. 13. Myxine no. 12 is 67 cm. and the testis band is 36 cm. long. Specimen no. 13 is 63 cm. long and its testis band measures 35 cm.

Transverse sections, cut in all parts of the testis bands of these two animals, reveal the fact that the bands are of uniform and peculiar structure. They do not resemble the testis bands of younger Myxine, nor those of *Bdellostoma*, as figures 80 and 81 show. The squamous mesorchial epithelium has been completely converted into the palisade layer of columnar cells. In transverse section the band is cone-shaped, and deep notches in the sides cut it into triangular lobes, leaving a central core of stroma. The latter has been entirely converted into fibrous connective tissue which contains many blood capillaries. Nowhere in the bands is there the slightest trace of primitive germ cells or follicles. Figure 81, a transverse section from the posterior end



of the band of Myxine no. 12, shows another peculiar condition. At the base of the band, in a pocket formed between the lobes, there is a tangled skein of columnar epithelium which is of the same width as the columnar epithelium around the band. Here and there this skein is continuous with the epithelium which envelopes the band. It extends through all the sections cut in the posterior region of the testis band of Myxine no. 12, but occurs nowhere else. The writer has been unable to explain what this tissue may be.

Myxine no. 12 and no. 13 are old animals, as is indicated by their general conditions, viz., the thick, hard skin, the reddish, coarse fibers of the muscular tissue, the enlarged, coarse, dark-brown liver, the great diameter of the body, etc. The peculiar condition of their testis bands is interpreted as due to their having become completely sterile with old age. Myxine no. 18 and no. 7, young, immature males, have many primitive germ cells, a few follicles and very little connective tissue in the testis band. Myxine no. 15, and adult male, has few germ cells, many follicles, and much connective tissue around and between the follicles in the testis lobes. In places the squamous epithelial covering of the band has been converted into a columnar epithelium, but sufficient squamous epithelium remains to permit easy rupture of the follicles when they are ripe. The band is notched only slightly here and there. In specimens no. 12 and no. 13 the germ cells and follicles have entirely disappeared, and the bands are completely filled with fibrous connective tissue and blood capillaries, and all the squamous mesorchial sheath has been converted into a columnar epithelium. The testis bands are deeply notched in all parts so that they are cut up into lobes. The testis bands in these two animals have lost their power of functioning and are completely sterile.

### *Bdellostoma*

*Female.* The generative organs of *Bdellostoma* closely resemble those of *Myxine* in many respects. The ovary is single, occurring on the right side only, and is suspended in the body

cavity from a mesovarium which extends from the region of the gall bladder to the posterior end of the body cavity, a distance which is approximately one-half the entire length of the body. Unlike Myxine, however, the proximal margin of the mesovarium is always attached to the mesentery about one-third the width of the latter from the alimentary canal. The mesentery and mesovarium are more delicate than in Myxine.

In the females which have eggs 2 mm. long or longer, the mesovarium is approximately 10 to 15 mm. wide. Where the large eggs are attached, the mesovarium is stretched into strands which are 25 to 30 mm. long, and between the eggs it is 10 to 20 mm. wide.

The distribution of the eggs is very similar to that in Myxine. There are all gradations of sizes of young eggs from mere dots to those 2 mm. long in adult females, but there are no intermediate sizes between the latter and any larger eggs or corpora lutea that may be present.

The following table will give some conception in regard to the number of large eggs which may occur in an adult female:

| NUMBER OF SPECIMEN | SIZE OF EGGS         | NUMBER OF EGGS |
|--------------------|----------------------|----------------|
|                    | <i>mm.</i>           |                |
| 25                 | 1.0-1.5 x 3.0-6.0    | 77             |
| 17                 | 2.5-3.0 x 10.0-11.0  | 20             |
| 1                  | 3.0-3.25 x 12.0-13.0 | 28             |
| 23                 | 3.0-3.5 x 13.0-14.0  | 10             |
| 24                 | 3.0-3.5 x 14.0-15.0  | 10             |
| 4                  | 3.0-3.5 x 14.0-15.0  | 23             |
| 15                 | 5.0-6.0 x 20.0-21.0  | 31             |

Two of the specimens of *Bdellostoma* contain corpora lutea, one having eleven, the other twenty-four, and all show signs of degeneration. Small brown ovals, representing degenerated eggs, are scattered irregularly among the eggs.

In all the females, the eggs extend to within 1 or 2 cm. of the posterior end of the mesovarium (fig. 84), and there are sometimes a few very small eggs in this most posterior part. No testis

lobes are present in any part of the mesovarium of any of the females, not even in the posterior region.

*Male.* No female elements occur in any of the males, and none of the mesorchia presents the appearance of ever having contained eggs. The general structure and appearance of the mesorchium is very similar in all the specimens. It extends from the region of the gall bladder to the caudal end of the body cavity, and is attached to the mesentery along a line which is about one-third the width of the latter from the alimentary canal (fig. 85). At the anterior end the mesorchium is merely a line along the dorsal surface of the digestive tract. At a point opposite the posterior end of the gall-bladder it leaves the alimentary canal and gradually ascends in the mesentery to its definitive position. Immediately behind the gall-bladder it is from 1 to 3 mm. wide, and retains this width to the testis lobes at the posterior end, usually decreasing slightly in width just before reaching the lobes. In most of the males the mesorchium, which is very delicate, contains no genital elements in the anterior and mid-regions, but in some individuals very small testis lobes are scattered along the distal margin of the mesorchium from the extreme anterior end to the band of lobes at the posterior end. One male has a single lobe about 0.5 mm. in diameter at the anterior end of the mesorchium and no others excepting those in the testis band at the posterior end. Other specimens have two or three such small lobes, still others a dozen or more, while one animal has so many that they look as though at one time they formed a continuous narrow band along the distal margin of the mesorchium.

The large mass of testis lobes is confined to the posterior end of the mesorchium, and consists of a great many lobules closely crowded together into a band, which varies in length, in the seven males examined, from 3 to 8 cm. and in width from 2 to 7 mm. There is no correlation between the length of the testis band and the length of the animal, as is shown by the following table:

| SPECIMEN NUMBER | LENGTH     | TESTIS BAND |           |
|-----------------|------------|-------------|-----------|
|                 |            | Width       | Length    |
|                 | <i>cm.</i> | <i>mm.</i>  | <i>cm</i> |
| 6               | 33.0       | 2           | 5.5       |
| 10              | 36.0       | 2           | 5.0       |
| 21              | 36.5       | 2           | 6.0       |
| 10              | 36.5       | 5           | 3.5       |
| 1               | 38.5       | 5           | 4.5       |
| 2               | 40.5       | 7           | 6.5       |
| 8               | 42.0       | 5           | 7.5       |

In the longer, i.e., the older, animals the testis band is wider and thicker than in the younger males.

The testis bands are folded more or less in the young males and considerably in the older animals. In all, the bands have a very granular appearance (fig. 85). The bands in the younger males are light gray in color, and yellowish in older animals.

Transverse sections of the testis bands show that in minute structure they are very similar to those of *Myxine*. Single germ cells and follicles of all sizes, from very small to large, are present. In a single section all stages of mitotic figures may occur, and many of the follicles contain spermatozoa (fig. 79). Each follicle has for its wall a single row of stroma cells, arranged end to end, and outside this are two or more concentric layers of fibrous connective tissue. Capillaries occur here and there in the connective tissue and stroma between the follicles. The primitive germ cells are limited to the distal end of the band, and here the follicles are smaller, their walls are not so prominent and there is less connective tissue than in the proximal part, where the large follicles are located. The entire band is covered by a single layer of squamous epithelium which is a continuation of that of the mesorchium. In no place is there columnar epithelium around the testis band as is described for *Myxine*.

Cunningham ('87) and Nansen ('87) have advanced the theory that *Myxine* is a protandric hermaphrodite, functioning as a male when young (28 to 32 cm. long) and as a female when older

(32 to 35 cm.). Nansen thinks the change in sex occurs when the animal reaches a body length of about 32 or 33 cm. Out of hundreds of specimens examined by each of these investigators, only a few males were found (Cunningham 8, Nansen 'very few') and most of these were immature. Nansen regards the males as merely transformed hermaphrodites and found all transition stages between males and common hermaphrodites. Cunningham found that in "nearly all specimens with very immature eggs the posterior portion of the sexual organ had the same structure as the testis," and that "in all specimens with well-developed ovarian eggs . . . with one exception, no testicular portion was present in the sexual organs," then he concluded "that in the young state the females are nearly, but not quite always hermaphrodite, and that the testicular portion normally disappears as the eggs become more mature." Nansen states that "on opening large specimens of *Myxine*, we generally find well developed ova in their sexual organs. If we, however, take smaller specimens, of about 28 to 32 cm. in length, and examine their sexual organs, we generally find that the anterior portion is but slightly prominent, and contains very small and young ova, whilst the posterior portion is often very broad and prominent, is lobate, and has a distinct whitish colour along its margin, and has, in all respects, the appearance that we would expect to find in a testis; and that it really is." He concludes that "*Myxine* is generally, or always (?), in its young state, a male; whilst at a more advanced age it becomes transformed into a female. Indeed, I have not yet found a single female that did not show traces of the early male stage."

Schreiner found only 19 pure males out of 2500 specimens examined, and they were seldom over 33 cm. long, the majority of them being 30 to 31 cm. In a quite preponderating number of individuals which were longer than 33 cm. there was a more or less well-developed ovary and the testis part was sterile, and in the males there was a more or less well-developed testis and an abnormal or rudimentary ovary. In the females, the ovary was well-developed and the testis part was usually rudimentary or occasionally completely sterile. The testis was always ab-



normal in both young and old females. In addition to functional males and females, Schreiner found individuals in which both testis and ovary were degenerated, and these he classed as sterile animals. He also found normal females 22 cm. long and less, and states that, "lässt sich das häufige Vorkommen von jungen Weibchen unmöglich mit der Annahme eines protandrischen Hermaphroditismus bei diesem Tiere vereinigen." Schreiner believes that *Myxine* is dioecious.

It is well known that *Bdellostoma* is an animal of separate sexes. The fifteen adult females examined by the writer have no trace of testis lobes in any part of the mesovarium, and no female elements occur in the mesorchium of any of the eight adult males. The females range in length of body from 34 to 40 cm., and the males from 36 to 42 cm. That is to say, in this particular lot of animals some of the functional males are larger than the adult females, hence it is certain that *Bdellostoma* is not a protandric hermaphrodite. It is improbable, to say the least, that of two animals so closely related as *Bdellostoma* and *Myxine*, the one should have normal, separate sexes while the other should possess such a peculiar method of reproduction as protandric hermaphroditism.

Furthermore, as Dean ('99) has suggested, the occurrence of large males would also discredit the theory that *Myxine* is a protandric hermaphrodite. *Myxine* no. 15, described above, is undoubtedly a normal male, in which the only female sexual products are small, completely degenerated eggs, and specimens no. 12 and no. 13 are males which have become sterile with age and which have no female elements whatever. *Myxine* no. 15 is 62 cm. long, no. 12 is 67 cm., and no. 13 is 63 cm. The average length of the females with large eggs, from 18 to 25 mm. long, is 59 cm., the longest being 64 cm. Here, then, we have one normal and two sterile males which are longer than the average female with large eggs. In view of these facts, it seems impossible that *Myxine* can be a protandric hermaphrodite.

## SUMMARY

1. A central duct is present in the pronephros of both *Bdellostoma* and the North American *Myxine*, but it is in a state of degeneration in both animals. This degeneration is more advanced in *Myxine* than in *Bdellostoma*, so that it includes the inner ends of the pronephric tubules and all parts of some of the largest tubules.

2. The 'central mass,' which lies in the pronephric vein, is not a series of glomeruli, but represents the disintegrated central duct (including the inner ends of tubules in *Myxine*).

3. The Malpighian body of the pronephros in both *Bdellostoma* and *Myxine* is located at the posterior end of the pronephros, and in appearance and structure closely resembles the Malpighian bodies of the mesonephros. It is formed by the fusion of two or more glomeruli.

4. The glomerulus of the pronephros in young *Myxinoids* is exposed to the pericardial cavity through a large opening, thus resembling a glomus. This opening becomes constricted to a small tubule in adult animals.

5. The large vein which extends along the base of each pronephros communicates with the lumen of the central duct through large natural openings. The pronephric tubules connect the lumen of the duct with the pericardial cavity, and the latter opens into the peritoneal cavity through the large pericardoperitoneal foramen, while the body cavity opens into the cloaca through the genital pore. Thus, the vascular system is in communication with the exterior of the body.

6. The morphology of the pronephros does not reveal any positive indications as to its function. The granular coagulum in the tubules and duct suggests excretion. This question can probably be determined only by physiological methods.

7. The tubules of the pronephroi of adult *Bdellostoma* are approximately the same diameter, while those of *Myxine* vary in size.

8. Each tubule in the pronephros does not represent an original pronephric tubule.

9. The mesonephric ducts of Myxinoids probably possess a secretory as well as an excretory function, as is suggested by the long epithelial cells, the increased surface of these by ridges, the large granules at the distal ends of the cells, and the yellowish bodies in the cells. Also, isolated pieces of the duct contain waste particles like those present in all parts of the continuous portion of the duct.

10. The mesonephric ducts of adult male *Bdellostomae* are larger than those of adult females, and the epithelial ridges within them are larger.

11. The mesonephric ducts of Myxinoids open separately into the cloaca.

12. The tubules of the mesonephric Malpighian bodies in *Bdellostoma* are structurally of two types.

13. When some eggs in the ovary of Myxinoids attain a size exceeding about 2 mm. in length, all the smaller eggs are arrested in growth until the large eggs reach maturity and have been passed from the body.

14. Many of the small, young eggs in Myxinoids degenerate, forming the 'brown bodies.'

15. When a mature egg passes from the ovary it leaves attached to the mesovarium a 'corpus luteum,' composed of the outer envelope of mesovarium which surrounded the egg. The corpora lutea degenerate.

16. After the testis of old males ceases to function, the peritoneal epithelium which covers the testicular band becomes changed from squamous to columnar; the empty testicular follicles completely disappear, and the band becomes converted into a mass of connective tissue penetrated by blood vessels.

17. Neither *Bdellostoma* nor *Myxine* is a protandric hermaphrodite.

18. The urogenital system of the North American *Myxine* does not present any specific differences from that of the European animal. On the other hand the great difference in size of mature individuals from the two sides of the Atlantic would appear to support the distinctness of *M. limosa* of Girard from the *M. glutinosa* of Linnaeus.

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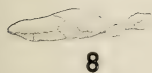
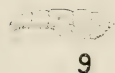
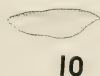
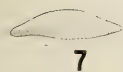
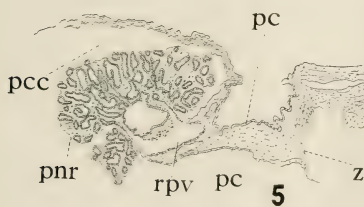
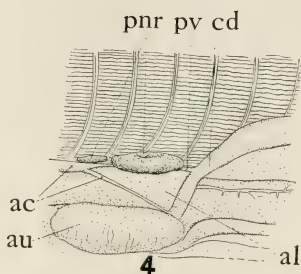
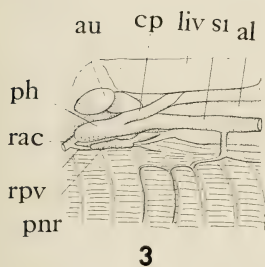
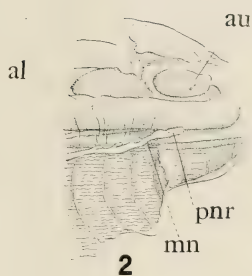
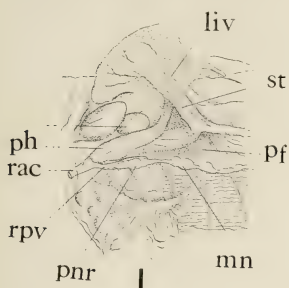
## ABBREVIATIONS

|  |   |
|--|---|
| <i>a</i> , dorsal aorta  | <i>mi</i> , mesoderm of peritoneum                                      |
| <i>ac</i> , anterior cardinal vein                               | <i>mn</i> , mesonephros   |
| <i>al</i> , alimentary canal                                     | <i>mp</i> , Malpighian body of pronephros                               |
| <i>an</i> , anus   | <i>mr</i> , mesorchium  |
| <i>au</i> , auricle  | <i>ms</i> , mesovarium  |
| <i>b</i> , Bowman's capsule                                      | <i>mt</i> , tubule of Malpighian body                                   |
| <i>bc</i> , trunk of compound tubule                             | <i>n</i> , nucleus  |
| <i>bm</i> , basal membrane                                       | <i>nc</i> , neck of compound mesonephric tubule                         |
| <i>bs</i> , blood sinus  | <i>nu</i> , nuclear mass  |
| <i>c</i> , connective tissue                                     | <i>o</i> , ovary  |
| <i>cb</i> , band of connective tissue                            | <i>op</i> , opening of central duct into pronephric vein                |
| <i>cd</i> , central duct   | <i>ov</i> , ova   |
| <i>ce</i> , columnar epithelium                                  | <i>p</i> , plain mesonephric tubule                                     |
| <i>cl</i> , corpora lutea  | <i>pc</i> , pericardium   |
| <i>clo</i> , cloaca  | <i>pcc</i> , pericardial cavity   |
| <i>cm</i> , central mass   | <i>pf</i> , pericardo-peritoneal foramen                                |
| <i>co</i> , blood corpuscle                                      | <i>pg</i> , primitive germ cell   |
| <i>cp</i> , common portal vein                                   | <i>ph</i> , portal heart  |
| <i>d</i> , distal margin of mesovarium or mesorchium             | <i>pn</i> , pronephros  |
| <i>dc</i> , ductus Cuvieri                                       | <i>pnr</i> , pronephros   |
| <i>dm</i> , degenerated Malpighian body                          | <i>pl</i> , epithelium of peritoneum                                    |
| <i>do</i> , degenerated ova, 'brown bodies'                      | <i>pv</i> , pronephric vein   |
| <i>e</i> , connective tissue envelope                            | <i>ra</i> , renal artery  |
| <i>eb</i> , epithelium of base of compound tubule of mesonephros | <i>rac</i> , right anterior cardinal vein                               |
| <i>el</i> , epithelium of plain mesonephric tubule               | <i>rp</i> , right postcardinal vein                                     |
| <i>em</i> , epithelium of mesonephric duct                       | <i>rpv</i> , right pronephric vein                                      |
| <i>en</i> , epithelium of neck of compound mesonephric tubule    | <i>s</i> , stoma cells  |
| <i>eo</i> , endothelium  | <i>sa</i> , somatic artery  |
| <i>ep</i> , epithelium   | <i>sc</i> , skein of columnar epithelium                                |
| <i>f</i> , funnel-shaped mouth of pronephric tubule              | <i>se</i> , squamous epithelium   |
| <i>fl</i> , testicular follicle                                  | <i>si</i> , supra-intestinal vein                                       |
| <i>g</i> , glomerulus  | <i>so</i> , somite  |
| <i>gb</i> , gall bladder   | <i>ss</i> , sheath of stroma cells around testicular follicle           |
| <i>gc</i> , giant blood corpuscle                                | <i>st</i> , septum transversum  |
| <i>gp</i> , process composed of granules                         | <i>t</i> , pronephric tubule  |
| <i>i</i> , intertubular space                                    | <i>tb</i> , testis band   |
| <i>liv</i> , liver   | <i>tu</i> , mesonephric tubule  |
| <i>lp</i> , left postcardinal vein                               | <i>v</i> , blood vessel   |
| <i>m</i> , Malpighian body of mesonephros                        | <i>w</i> , waste granules   |
| <i>md</i> , mesonephric duct                                     | <i>y</i> , yellowish granules   |
|  | <i>z</i> , pericardial attachment to dorsal aorta and alimentary canal. |

## PLATE 1

### EXPLANATION OF FIGURES

- 1 Right pronephros of *Bdellostoma* no. 6, showing its position in the pericardial cavity. Natural size.
- 2 Left pronephros of *Bdellostoma* no. 6, showing position in the pericardial cavity. Natural size.
- 3 Right pronephros of *Myxine* no. 5, showing position in the pericardial cavity. Natural size.
- 4 Left pronephros of *Myxine* no. 5, showing position in the pericardial cavity.  $\times 1\frac{1}{2}$ .
- 5 Transverse section through the right pronephros of *Bdellostoma* no. 6. Camera,  $\times 25$ .
- 7 Left pronephros of *Bdellostoma* no. 16.  $\times 4$ .
- 8 Left pronephros of *Bdellostoma* no. 18.  $\times 4$ .
- 9 Left pronephros of *Bdellostoma* no. 7.  $\times 4$ .
- 10 Right pronephros of *Bdellostoma* no. 16.  $\times 4$ .
- 11 Right pronephros of *Bdellostoma* no. 18.  $\times 4$ .



## PLATE 2

### EXPLANATION OF FIGURES

11a Transverse section of the right pronephros of *Bdellostoma* no. 10, illustrating the three-sided shape.

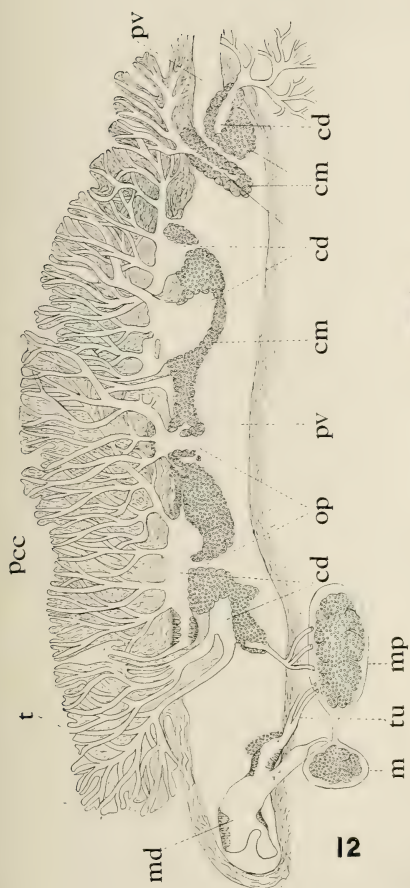
12 Millimeter paper reconstruction of the right pronephros of *Bdellostoma* no. 15.  $\times 25$ .

13 Pronephric tubule with funnel-shaped mouth.

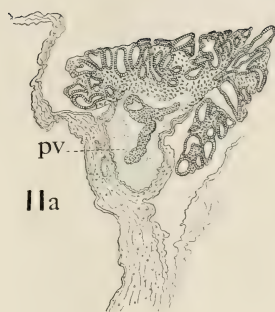
14 Pronephric tubules in longitudinal and transverse section. *Bdellostoma* no. 15.

15 Transverse section through two pronephric tubules showing relation of the vascular endothelium to the tubules when a blood sinus occupies the space between the tubules. *Bdellostoma* no. 15.

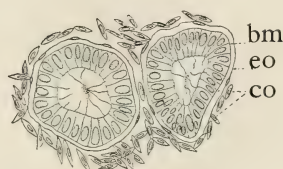




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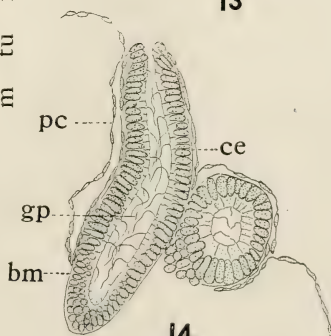
11a



15



13



14

### PLATE 3

#### EXPLANATION OF FIGURES

16 Enlarged drawing of columnar epithelial cells of a pronephric tubule. *Bdellostoma* no. 15.

17 Transverse section through pronephros of *Bdellostoma* no. 10, showing the endothelium of the pronephric vein continuing around the central mass.

18 Transverse section through the central mass. *Bdellostoma* no. 15.

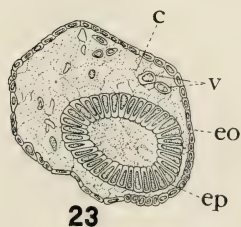
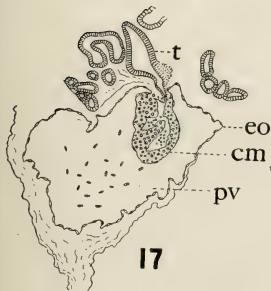
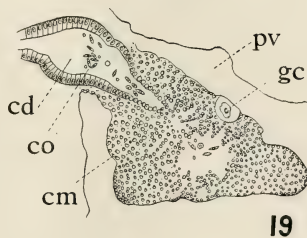
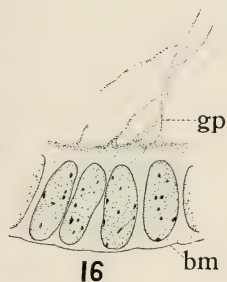
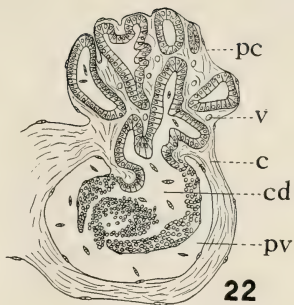
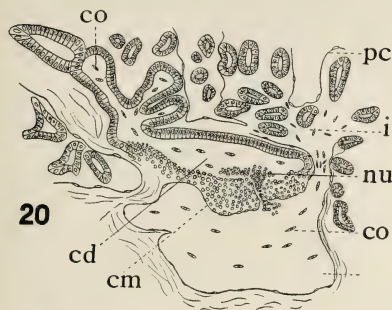
19 Enlarged drawing of the central mass. *Bdellostoma* no. 15.

20 Transverse section through pronephros of *Bdellostoma* no. 15, showing communication between the central duct and the pronephric vein, with nuclear mass in the opening; also shows pronephric vein opening into the space between the tubules, thus making a blood sinus. Not all the pronephric tubules are included in the drawing.

21 Transverse section in another part of pronephros of *Bdellostoma* no. 15, which also shows the central duct communicating with the pronephric vein and the opening filled with the nuclear mass. Not all of the tubules are shown.

22 Transverse section through pronephros of *Bdellostoma* no. 6, showing the central duct in communication with the pronephric vein through a large opening which is filled with a nuclear mass.

23 Transverse section through a pronephric tubule of *Myxine* no. 10, showing connective tissue surrounding the epithelium.



## PLATE 4

### EXPLANATION OF FIGURES

24 Transverse section through a pronephric tubule of *Myxine* no. 11, showing connective tissue.

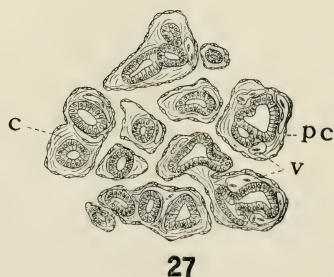
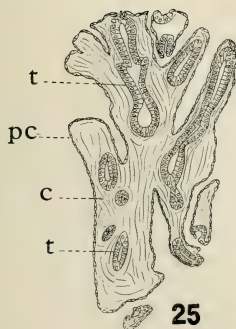
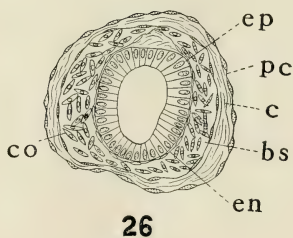
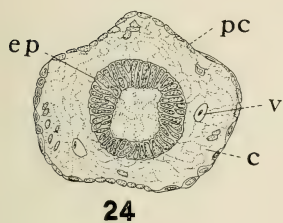
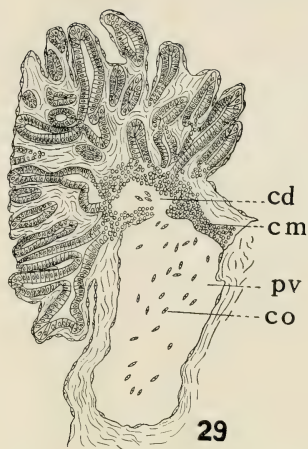
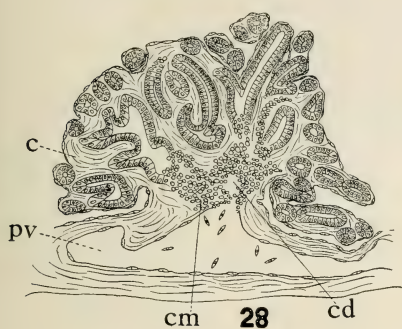
25 Section through some tubules of pronephros of *Myxine* no. 15, showing the large amount of connective tissue around the tubules.

26 Transverse section through a pronephric tubule of *Myxine* no. 10, showing blood sinus around the tubule.

27 Transverse section through some tubules of pronephros of *Myxine* no. 10, showing difference in size of the tubules.

28 Transverse section through pronephros of *Myxine* no. 11, showing the central duct in the central mass.

29 Transverse section through pronephros of *Myxine* no. 11, showing central duct in communication with the pronephric vein. Camera lucida,  $\times 50$ .





## PLATE 5

### EXPLANATION OF FIGURES

30 Longitudinal section through the central duct in pronephros of Myxine no. 11. Camera lucida,  $\times 50$ .

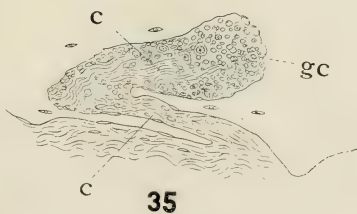
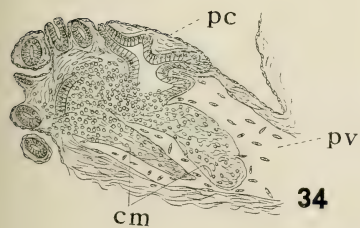
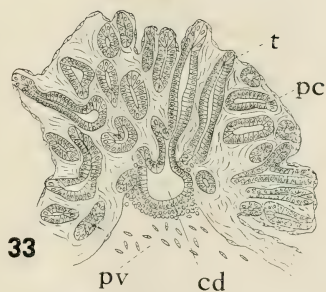
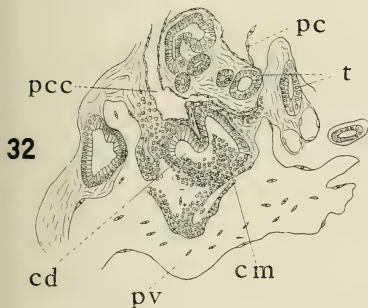
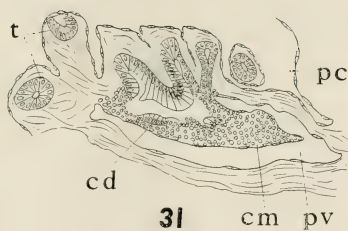
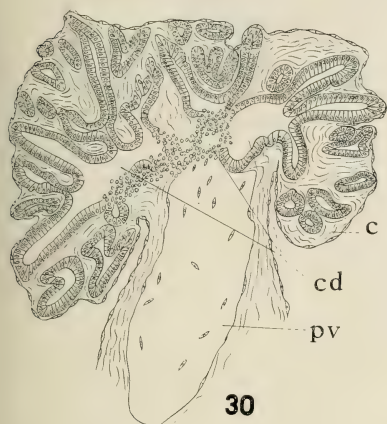
31 Longitudinal section through the central duct of one of the youngest specimens, Myxine no. 18. Camera,  $\times 90$ .

32 Transverse section through pronephros of Myxine no. 10, showing the central duct. Not all the tubules are included in the sketch. Camera lucida,  $\times 30$ .

33 Transverse section through pronephros of Myxine no. 11, showing the central duct with its columnar walls intact. Camera lucida,  $\times 50$ .

34 Transverse section through pronephros of one of the youngest specimens. Myxine no. 18, showing the central mass. Camera lucida,  $\times 50$ .

35 Longitudinal section through the central mass in pronephros of Myxine no. 11. Camera lucida,  $\times 90$ .



## PLATE 6

### EXPLANATION OF FIGURES

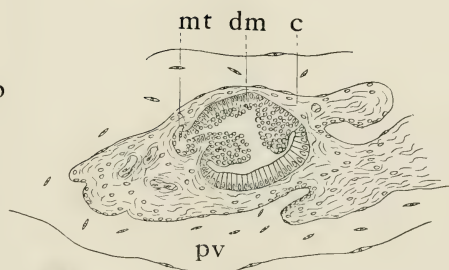
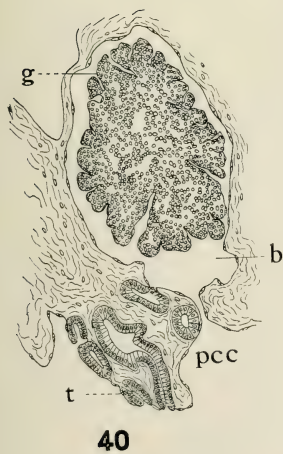
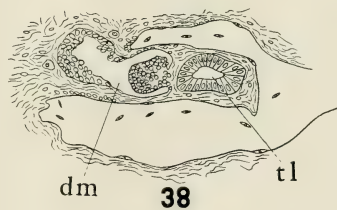
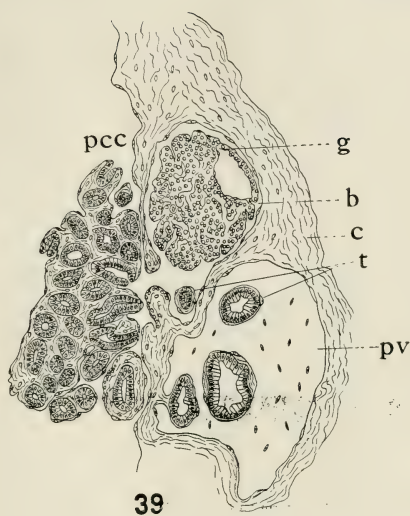
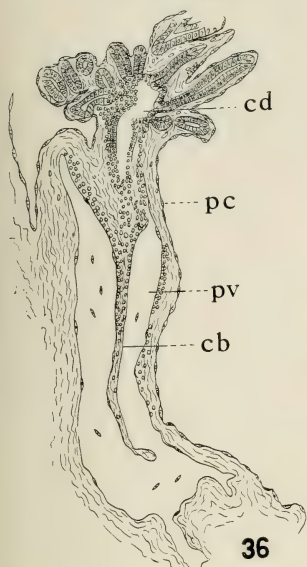
36 Section pronephros of *Myxine* no. 15 which shows a broad band of connective tissue extending from the base of the tubules into the pronephric vein. Camera,  $\times 50$ .

37 Transverse section through a degenerated Malpighian body immediately posterior to the left pronephros *Myxine* no. 15. Camera lucida,  $\times 90$ .

38 Transverse section through a degenerated Malpighian body immediately posterior to the left pronephros of *Myxine* no. 11, with a blind tubule beside it. Camera,  $\times 90$ .

39 Transverse section through pronephros of *Bdellostoma* no. 15, showing Bowman's capsule of the Malpighian body opening into the pericardial cavity. Camera lucida,  $\times 50$ .

40 Transverse section through the Malpighian body of the left pronephros of *Bdellostoma* no. 16, showing lobulated structure of the glomerulus and the opening of Bowman's capsule into the pericardial cavity. Camera,  $\times 90$ .



## PLATE 7

### EXPLANATION OF FIGURES

41 Section through the Malpighian body of one of the oldest specimens (Myxine no. 10), showing degeneration of the glomerulus. Camera lucida,  $\times$  19.

42 Section through the Malpighian body of pronephros of a young Myxine (no. 18), showing double glomerulus. Camera lucida  $\times$  50.

43 Section through Malpighian body of pronephros of a young Myxine (no. 18), showing the glomerulus exposed to the pericardial cavity through a large opening and resembling a glomus. Camera lucida,  $\times$  50.

44 Transverse section through mesonephros of *Bdellostoma* no. 17, cut in mid-region of body cavity, showing relation of the ducts to blood vessels and peritoneum.

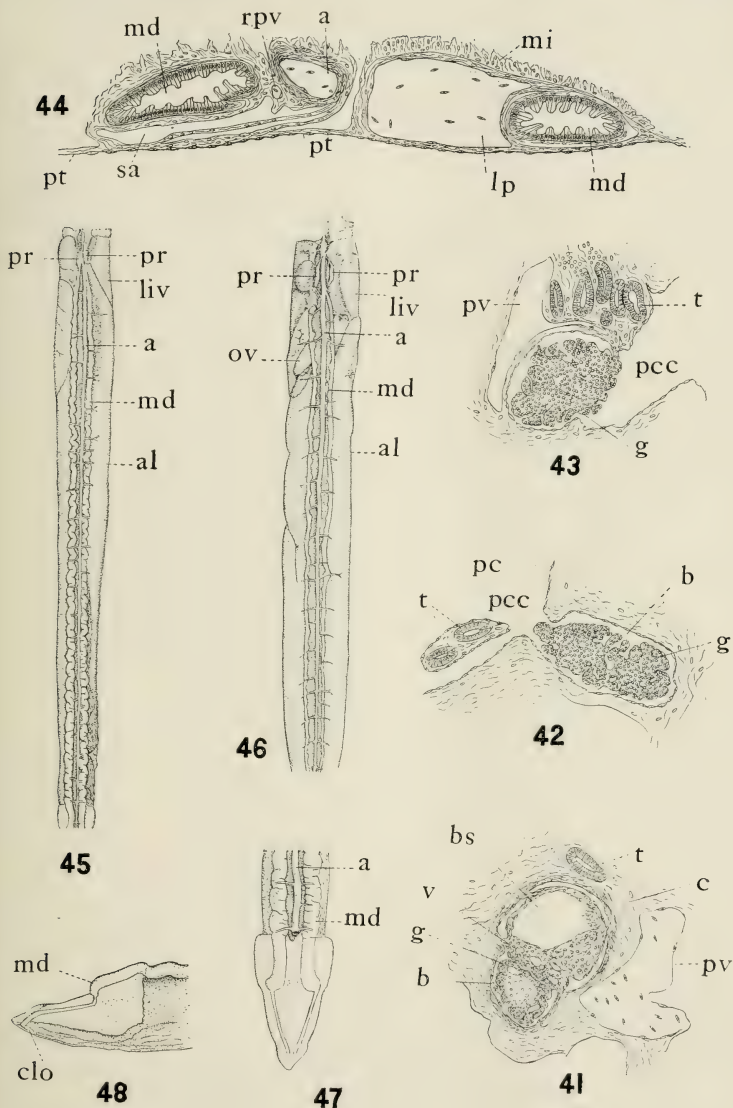
45 Dorsal view of the excretory system of *Bdellostoma* no. 16. One-half natural size. Male.

46 Dorsal view of the excretory system of *Bdellostoma* no. 15. One-half natural size. Female.

47 Dorsal view of posterior ends of mesonephric ducts of *Bdellostoma* no. 16. Natural size.

48 Side view of posterior end of left mesonephric duct of *Bdellostoma* no. 16. Natural size.





## PLATE 8

### EXPLANATION OF FIGURES

49 Ventral view of excretory system of a young male, *Bdellostoma* no. 6. One-half natural size.

50 Transverse section through the mesonephric duct of an adult male, *Bdellostoma* no. 16, cut in the mid-region of the body cavity. Camera lucida,  $\times 19$ .

51 Transverse section through the mesonephric duct of an adult female, *Bdellostoma* no. 15, cut in the mid-region of the body cavity. Camera lucida,  $\times 19$ .

52 Transverse section through the anterior end of a mesonephric duct of *Bdellostoma* no. 6,  $\times 90$ .

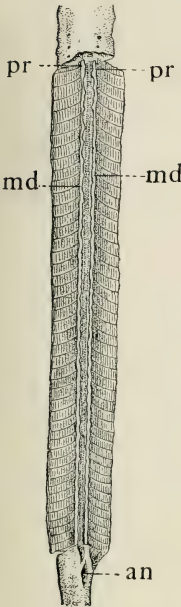
53 Enlarged sketch of a ridge in the epithelial lining of a mesonephric duct. *Bdellostoma*.

54 Enlarged sketch of the anterior ends of the mesonephric ducts of *Bdellostoma* no. 4, mounted in damar.

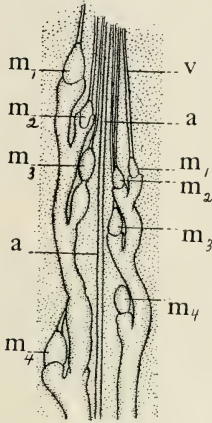
55 Enlarged ventral view of the mesonephric ducts of *Bdellostoma* no. 8, showing location of the Malpighian bodies with reference to the somites.

56 Enlarged sketch of mesonephric ducts of *Bdellostoma* no. 16 showing the distribution of the arteries to the Malpighian bodies.

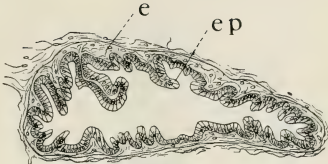
57 Longitudinal section through the tubule of a Malpighian body of the mesonephros, *Bdellostoma* no. 17. 'Plain' type of tubule. Camera lucida,  $\times 90$ .



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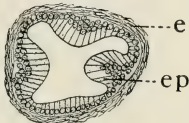
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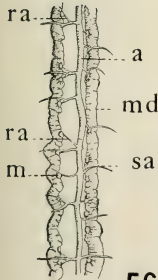
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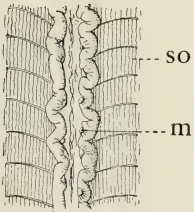
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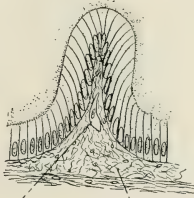
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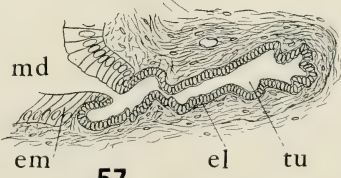
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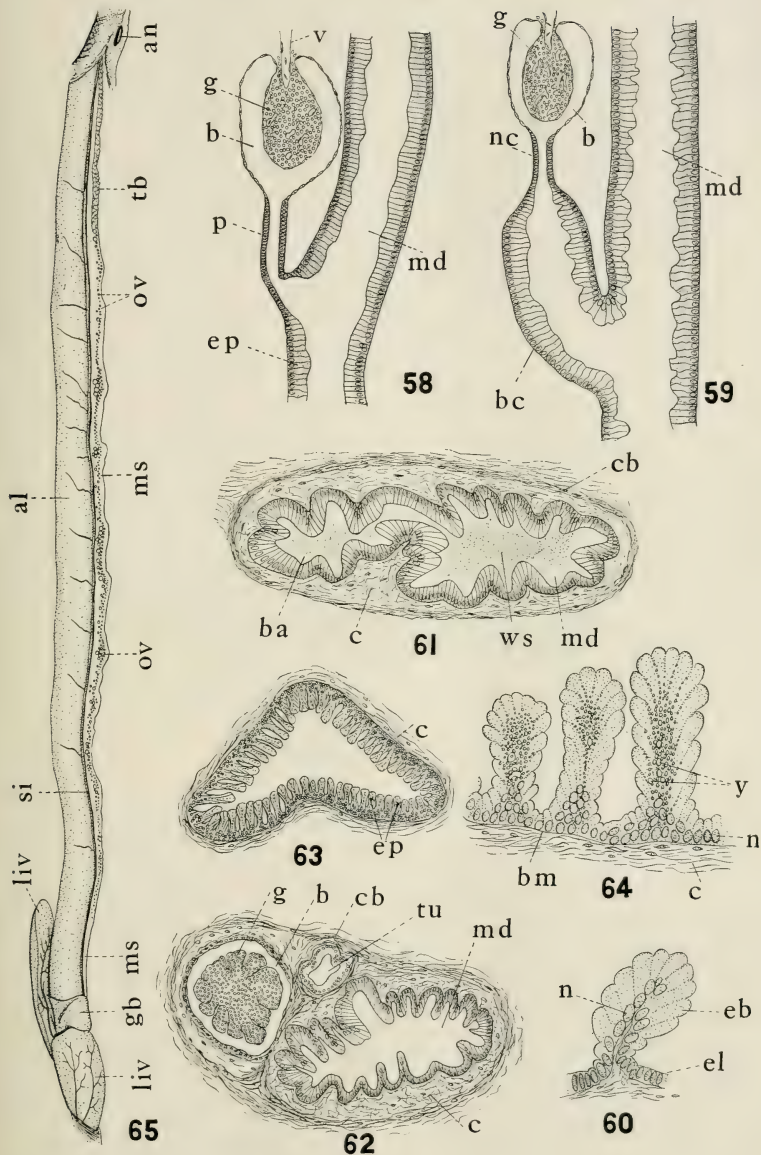


57

## PLATE 9

### EXPLANATION OF FIGURES

- 58 Reconstruction of a 'plain' mesonephric tubule of *Bdellostoma*; diagrammatic.
- 59 Diagrammatic reconstruction of a 'compound' mesonephric tubule of *Bdellostoma*.
- 60 Sketch showing transition between the cells of the neck and those of the trunk in a 'compound' tubule.  $\times 250$ .
- 61 Transverse section of a 'compound' tubule showing opening of the trunk into the mesonephric duct. Camera,  $\times 50$ .
- 62 Transverse section through mesonephric duct of *Bdellostoma* no. 15, showing a Malpighian body and its tubule. Camera lucida,  $\times 30$ .
- 63 Transverse section through mesonephric duct of *Myxine* no. 12. Camera lucida,  $\times 50$ .
- 64 Enlarged sketch of epithelial ridges in mesonephric duct of *Myxine* no. 12, showing core of yellowish granules.
- 65 Sketch of ovary of one of the youngest specimens of *Myxine* (no. 18). Natural size.

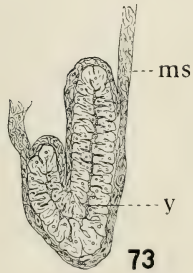
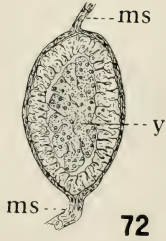
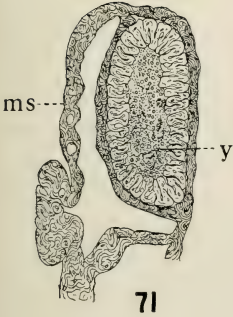
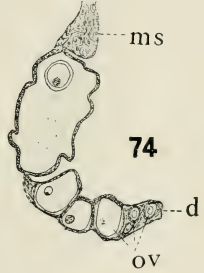
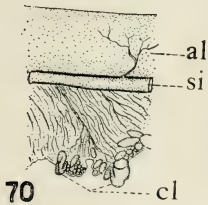
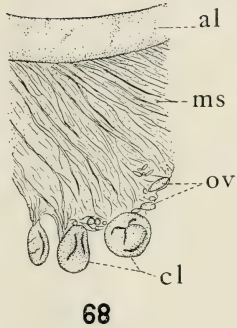
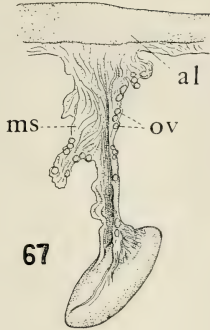
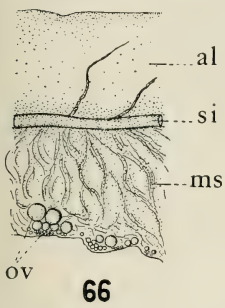




## PLATE 10

### EXPLANATION OF FIGURES

- 66 Enlarged portion of ovary of an adult Myxine (no. 16), showing distribution of eggs.  $\times 2$ .
- 67 Sketch of large egg in Myxine no. 2. Natural size.
- 68 Portion of mesovarium containing 'corpora lutea.' Myxine no. 20. Natural size.
- 69 A large corpus luteum from Myxine no. 2. Natural size.
- 70 Small corporea lutea from Myxine no. 5. Natural size.
- 71, 72, and 73 Sections of 'brown bodies' from Myxine showing three stages in the degeneration of eggs. Camera lucida,  $\times 30$ .
- 74 Section through small eggs from Myxine no. 8. Camera lucida,  $\times 30$ .



## PLATE 11

### EXPLANATION OF FIGURES

75 Transverse section through the testis band at the posterior end of the mesovarium of a young *Myxine* (no. 18). Camera lucida,  $\times 50$ .

76 Transverse section through the testis band at the posterior end of the mesovarium of an older, but immature *Myxine* (no. 7). Camera lucida,  $\times 30$ .

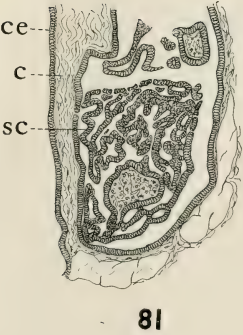
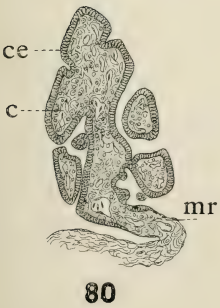
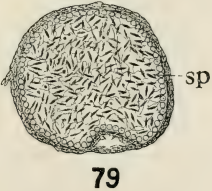
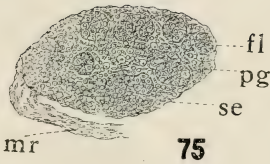
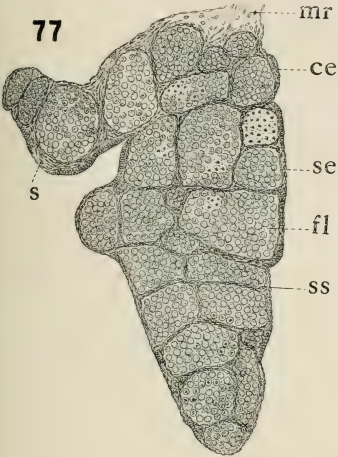
77 Transverse section through the testis band of *Myxine* no. 15, a normal male. Camera lucida,  $\times 50$ .

78 Enlarged sketch of a transverse section through a follicle in the testis of *Myxine* showing the spermatogonia in the metaphase stage.

79 Transverse section through a follicle in the testis band of *Bdellostoma* no. 7, showing spermatozoa.

80 Transverse section through the anterior region of the testis band of *Myxine* no. 12. Camera lucida,  $\times 30$ .

81 Sketch of a portion of transverse section through the posterior region of the testis band in *Myxine* no. 12. Camera lucida,  $\times 30$ .

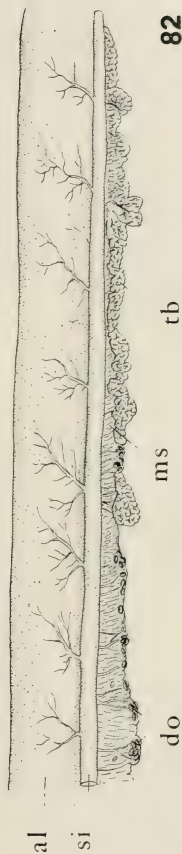
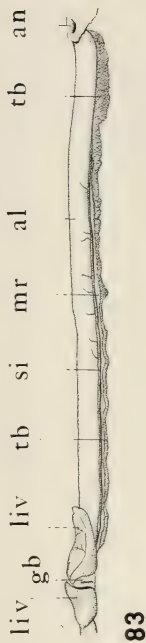
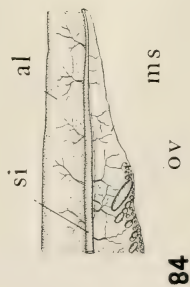
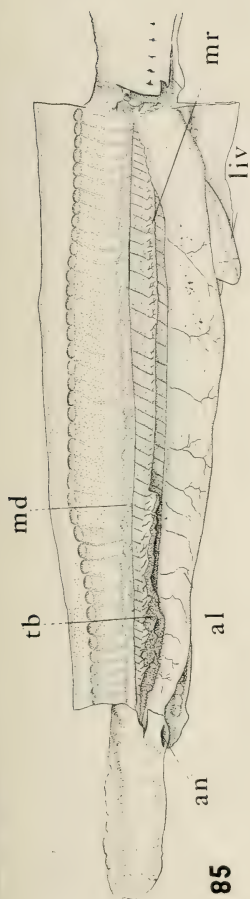


## PLATE 12

### EXPLANATION OF FIGURES

- 82 Sketch of the posterior end of the genital band in *Myxine* no. 15. Natural size.
- 83 Sketch of the testis band in *Myxine* no. 12. One-fourth natural size.
- 84 Sketch showing extreme posterior end of the genital band in a female *Bdellostoma* (no. 9). Natural size.
- 85 Sketch of a male *Bdellostoma* (no. 8) with body cavity open, showing the testis band. One-half natural size.







# STUDIES ON THE SYRINX OF GALLUS DOMESTICUS

JAY ARTHUR MYERS

*Department of Anatomy, University of Minnesota*

EIGHTEEN FIGURES

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## INTRODUCTION

The songs of birds have served to inspire various classes of people for ages, but not until the last three or four centuries do we have any record of scientists becoming interested in them to the extent of studying the organs concerned in their production.

Competent authorities, such as Duvernoy, Cuvier, Johannes Müller, and others, early observed that birds possess two larynges

—an upper and a lower. From their observations these early investigators concluded that it was in the lower larynx that the voice is produced. Regarded as a voice organ, the syrinx has aroused much interest and has been the subject of numerous researches. These researches deal chiefly with its function, structure, and comparative anatomy. Its embryology has been quite neglected, and it was this fact that prompted the present investigation. At the outset it was thought best to confine this study to some single species of bird, and to work out the development of the voice organ, stage by stage, in this one form. It was found, however, that many anatomical details of the syrinx and its related structures had also been neglected. Therefore, before the embryological study could be undertaken, it became necessary to inquire more minutely into its anatomy. Somewhat incidentally the function of the syrinx, too, has been considered, and experiments performed to determine whether the lower larynx is, in truth, the voice-producing organ.

This investigation was undertaken on the suggestion of Prof. H. D. Reed of Cornell University, and was carried on, under his supervision in the Laboratories of Comparative Anatomy at that University until September, 1913, when it was transferred to the Anatomical Laboratories of the University of Missouri. To Dr. Reed of the former university the author is deeply indebted for many valuable suggestions and criticisms. At the latter university, the author is obligated to Dr. F. P. Johnson for his keen interest in the embryological division of these studies, and to Thos. J. Heldt for his aid and criticism.

For the early literature the reader is referred to L. Wunderlich ('84), who gives an excellent historical sketch of the work previous to his own. He includes a very complete résumé of all work dealing with the syrinx as studied in numerous species of birds.

In his text book entitled "The development of the chick" Lillie '08, treats the respiratory system quite fully, but states that very little is known concerning the development of the voice organ in the chick.

## OBSERVATIONS

In this investigation *Gallus domesticus* was used for two reasons. First, adults are accessible at all seasons of the year. Second, developmental stages are easily obtained and controlled.

## I. ANATOMY OF THE ADULT SYRINX

*1. Gross anatomy*

Birds differ from mammals in possessing two larynges, an upper and a lower. The latter, known also as the syrinx, was described by Johannes Müller as occurring in three different positions. When the organ is located at the lower end of the trachea but above the bifurcation, he designated it 'syrinx trachealis.' If found in the bronchi below the bifurcation, 'syrinx bronchialis.' But if the lower end of the trachea and the upper portions of the bronchi both took part in its formation he called it 'syrinx tracheo-bronchialis' (fig. 2). The syrinx of the domestic chicken belongs to the last type.

In the domestic chicken, the upper larynx (fig. 1) is located behind the tongue and on the floor of the pharynx. It is rudimentary when compared with that of other animals and is apparently incapable of producing voice. From the upper larynx the trachea courses caudad, ventral to the oesophagus, passes to the left of the ingluvies and pierces the pleura between the limbs of the furcula to enter the so-called thoracic cavity. Shortly after entering this cavity, the trachea bifurcates into the two primary bronchi. In the region of the bifurcation a great modification of the cartilaginous and intercartilaginous elements takes place. Here (fig. 2) a well developed bone, known as the cross-piece, or pessulus, lies transversely in the dorso-ventral plane between the two bronchi. Its dorsal and ventral extremities are greatly enlarged. The caudal end of the trachea is compressed. It is composed of rings which are enlarged and closely related to each other. The four most caudal rings are imperfectly fused. Immediately caudad to this fusion are four



highly modified cartilages which have free ends dorsally, but fuse with the pessulus ventrally.

The cartilages of the bronchi, unlike those of the trachea, are present only as halves of rings. These half-rings form the framework of the lateral bronchial walls. The first half-rings are modified, and are attached to the pessulus both dorsally and ventrally. The second are only slightly modified and are attached ventrally to the first by a small piece of cartilage. Their dorsal ends are free. Certain of these skeletal elements, so briefly referred to, serve as attachments for vibrating membranes. The external tympanic membranes (fig. 2) stretch between the first tracheal ring and the first half-ring. First, as used here in reference to the tracheal rings, designates the most caudal, as regards an enumeration of the total number of tracheal rings in the caudo-cephalic direction. First, as used here in reference to the bronchial half-rings, has a somewhat different meaning. It designates that bronchial half-ring which of all the bronchial half-rings lies in the most cephalic position. These designations are consistently adhered to throughout the paper. The internal tympanic membranes form that part of the medial wall of each primary bronchus which lies immediately caudad to the pessulus. They stretch, therefore, from the pessulus to the cephalic end of the medial membranous wall of each primary bronchus. In fact, the internal tympanic membranes may be regarded as specialized portions of the medial membranous walls. Still more caudad to the pessulus and between the caudal extremities of the two internal tympanic membranes, stretching transversely in the medio-lateral plane, is another membrane, the bronchidesmus. From its position, then, the bronchidesmus connects the medial walls of the two bronchi.

*a. Skeletal framework.* In the study of the bony and cartilaginous skeleton, three methods were pursued. First, numerous gross dissections were made. Second, cartilage and bone stains were used to stain the organ in toto. For staining bone 2 per cent alizarine in 96 per cent alcohol was used, while  $\frac{1}{4}$  per cent methylene blue in 67 per cent alcohol was employed as a cartilage

stain. These organs were then washed and cleared, after which the cartilaginous and the bony structures could be more easily studied with the binocular microscope. Owing, however, to the size and the shape of the organ as a whole, this method was not entirely satisfactory. Third, wax reconstructions were made according to the method described by Born.

The syringeal skeleton (fig. 4) consists of (1) the first four tracheal rings which may be designated, the cephalic syringeal skeletal elements; (2) the first three bronchial half-rings, or the caudal syringeal skeletal elements; (3) four modified cartilages, neither rings nor half-rings, which are found between the tracheal rings and the first bronchial half-rings. These modified structures will be designated the intermediate syringeal cartilages; (4) a bony pessulus which lies at the summit of the bronchial junction in the dorso-ventral plane and transverse to the long axis of the trachea. Although the skeletal elements are similar in most respects in both sexes, the following description applies, unless stated otherwise, only to the male.

The typical tracheal rings are broad flat bands of cartilage. They differ from the type found in most of the higher animals in that they are complete rings. In cross section the typical rings have a cephalo-caudal diameter which is much greater than the medio-lateral diameter, hence they appear very much elongated (fig. 5). But as the caudal end of the trachea is approached, the cephalo-caudal diameter of the rings diminishes while the medio-lateral diameter increases. The fifth, the sixth, and the seventh rings appear almost square when seen in cross section. These, as well as all other rings cephalad of them, are transverse to the long axis of the trachea and constitute the framework of its walls. The medio-lateral diameter of the fourth ring is greater than its cephalo-caudal diameter. The most ventral portion of this cartilage dips caudad to a considerable extent.

The first four tracheal rings (fig. 4) are very closely related to each other. They are partially fused along their sides as well as firmly bound together by dense fibrous tissue. Their ventral and dorsal extremities are free, but the spaces between these

extremities are very narrow. This arrangement gives a very strong wall to this portion of the trachea, and because of this specialization this portion is known as the tympanum. The most caudal, or first, tracheal ring is smaller than the other three and is transitional in size and shape between the tracheal rings immediately above it and the intermediate syringeal cartilages immediately below it. As a whole, the tympanum arches distinctly cephalad, its ventral and dorsal extremities projecting caudad.

For the proper understanding of the arrangement of the remaining cartilages a description of the pessulus (fig. 3) here becomes necessary. In size, the pessulus far exceeds all other skeletal parts. It is a well developed bar of bone located at the junction of the bronchi, and lying dorso-ventrally in a plane transverse to the long axis of the trachea. Its ventral and dorsal extremities are large and serve for the attachment of some of the cartilages referred to above.

The bone as a whole may be described as consisting of a shaft and two large extremities (fig. 3). The shaft corresponds to a little more than the middle third of the bone and in shape resembles a three-sided prism with rounded borders. The cephalic porder projects into the lumen of the trachea, and marks the point of its bifurcation. The lateral surfaces of the shaft form the medial walls of the cephalic ends of the bronchi, between the diverging courses of which the basal surface lies.

The ventral extremity is very large and may be considered as being a pyramid whose apex points cephalad. On this pyramid three distinct borders, a dorsal and two lateral; three surfaces, a ventral and two dorso-lateral; and a base, may be described. The dorsal border is continuous with the cephalic border of the shaft and projects into the lumen of the trachea. The two lateral borders give attachment to the intermediate syringeal cartilages which will be described presently. The ventral or anterior surface is broad and slightly convex and stands out so prominently that when the syrinx is viewed as a whole, this surface projects farther ventrad than any immediately neighboring skeletal element. The caudal portions of the dorso-lateral sur-

faces, like the lateral surfaces of the shaft of the pessulus with which they are continuous, form the medial walls of the cephalic ends of the bronchi, while the cephalic portions of these same dorso-lateral surfaces form a part of the ventral wall of the trachea. The base, or basal surface, lies in the same plane as the corresponding surface of the shaft of the pessulus, and like the latter surface can be plainly seen in a view of the caudal aspect of the entire syrinx.

The dorsal extremity of the pessulus, like the ventral, is a three-sided pyramid with its apex pointing cephalad. A ventral border and two lateral borders, a dorsal surface and two ventro-lateral surfaces, and a base, or basal surface, are to be noted. With the exception of the dorsal surface, the surfaces and borders of this extremity have the same corresponding relations as those of the ventral extremity. The dorsal surface lies in close relation to the ventral wall of the oesophagus. To no part of this extremity, however, do the intermediate syringeal cartilages find attachment.

The first bronchial half-ring is attached at both ends to the lateral borders of the extremities of the pessulus. At its dorsal end this connection is composed of fibrous connective tissue. At the ventral end the union is made by means of cartilage. In old age, however, these attachments become ossified. In females of one or two years the half-rings are united to the ventral extremity of the pessulus by young cartilage which appears much lighter than other cartilaginous portions, when stained with hematoxylin.

The four cartilages which occur between the tympanum and the first bronchial half-rings (fig. 4), and which have been designated above as the intermediate syringeal cartilages, appear to differ structurally from all the other cartilages in the entire respiratory tract. They are not complete rings nor are they half-rings. Ventrally they are continuous with the pyramid of the pessulus, while dorsally they are free. The most cephalic ones are smallest and the most rudimentary. Ventrally they proceed from the very apex of the pyramid of the pessulus, extend along the sides of the syrinx as thin flat bands, and end before



reaching its dorsal wall. The second, enumerated caudad from the tympanum, are somewhat broader and extend farther dorsad. The first and the second cartilages are often fused for a part of their course. The third intermediate syringeal cartilages differ from the second only in being slightly broader. The fourth and the last of this series present more striking differences. They are larger than any of the preceding ones. Their free dorsal extremities lie laterad of the dorsal pyramid and gradually become very broad, but their medio-lateral diameter is not increased. These cartilages are arched slightly cephalad.

Caudad to the intermediate syringeal cartilages are the bronchial half-rings (fig. 4). The first of these differ from the typical half-rings in the following respects: first, they are longer and have much greater diameters; second, both ends are attached to the pessulus; third, well marked caudal prolongations extend from their ventral extremities and connect them with the second half-rings; fourth, their ventral extremities in old individuals become ossified; fifth, they are arched, with their convexities pointing caudad.

Between the first half-rings and the last intermediate cartilages are very large, somewhat oval-shaped spaces, the upper thirds of which are directly opposite to the pessulus (fig. 4).

The second half-rings, which are somewhat smaller than the first, have enlarged ventral extremities which are attached to the cartilaginous prolongations of the first half-rings mentioned above. Their dorsal extremities are free.

The third bronchial half-rings are quite similar to the typical half-rings which lie caudad to them. The only noticeable modification is an enlargement of their ventral extremities. They are somewhat flattened bands of cartilage which are free at both ends.

*b. Vibrating membranes.* Certain parts of the highly modified skeleton, just described, serve as attachments for specialized semitransparent membranes, which are the real voice producing elements. Two pairs of these are present in the chicken. They are known as the external and the internal tympanic membranes. Another membrane, not strictly a vibrating membrane, will also be discussed under this heading.



It should be recalled that the first half-ring of each primary bronchus and the last intermediate syringeal cartilage arch caudad and cephalad respectively, thus forming two (one on each side) oval spaces, occupied by the external tympanic membranes (fig. 2). Caudally these membranes are attached along the entire length of the cephalic borders of the first half-rings; cephalically they are attached along the entire length of the caudal borders of the last intermediate cartilages. Dorsally and ventrally the membranes narrow and are attached to the lateral borders of the corresponding pyramids of the pessulus. The internal tympanic membranes are situated more caudad (fig. 2), in the medial walls of the bronchi just below the tracheal bifurcation and stretch between the dorsal and ventral free ends of the half-rings. Cephalically they are attached to the lateral borders of the shaft of the pessulus. Caudally they extend to the level of the third half-rings where they are replaced by the heavy fibrous tissue of the medial bronchial walls.

Stretching across the interbronchial interval, just caudad to the internal tympanic membranes, is another membranous structure, the bronchidesmus (figs. 1 and 2) of Garrod. The middle portion of this is somewhat narrower than its ends, and may be said to lie between the level of the fifth and the level of the eighth bronchial half-rings. Its ends, exceeding these levels and extending for a variable distance above and below them, are attached correspondingly to the dorsal third of the medial bronchial walls. The cephalic portions of these attachments show a modification. As these portions of the ends of the bronchidesmus extend to the attachment stated, they expand, dorso-ventrally, and find further attachment as far ventral as the ventral third of the membranous medial walls of the bronchi and as far cephalad as the level of the third bronchial half-rings. This last attachment may be said to mark the caudal limits of the internal tympanic membranes. The caudal border of the bronchidesmus is broader than its cephalic border and presents two oval openings, one on each side of the median plane, which lead into two smaller irregular pockets within the bronchidesmus.

In the interbronchial region, in immediate relation to the caudal border of the bronchidesmus, numerous folds and pouches are developed. In the mid-line, the dorsal surface of the bronchidesmus is attached, by means of a thin sheet of fibrous tissue, to the ventral surface of the oesophagus and the dorsal portion of the cephalic part of the pericardium. From these attachments this same sheet of tissue extends ventrally, and is further attached to the caudal border of the bronchidesmus, which thus receives additional anchorage by the sheet of tissue also being attached to the ventral portion of the cephalic part of the pericardium. From these last named attachments the sheet of tissue extends cephalad in the median plane, and tapers to a narrow cord. The cord, after a short course, again expands, this time to gain attachment along the entire extent of the junction of the ventral surface and the base of the ventral pyramid of the pessulus. Ventrally this cord and its expanded portions are attached throughout the entire length to a reflection of the fibrous pericardium surrounding the roots of the great vessels of the heart.

*c. Musculature.* In general the musculature of the syrinx of birds is composed of intrinsic and extrinsic muscles. Many birds have from three to seven pairs of intrinsic syringeal muscles. The frequent occurrence of these intrinsic muscles has been recognized of value in the classification of birds. Species possessing them are usually songsters. There are some exceptions, however, such as the crow and the jay, which, though possessing these intrinsic muscles, are no longer classified as singing birds. From the syrinx of the domestic chicken the intrinsic syringeal muscles are absent, and hence its voice organ is of course correspondingly simpler. Because of their absence from the syrinx of *Gallus domesticus* the intrinsic syringeal muscles merit no further description here.

The principal extrinsic syringeal muscles are the tracheal muscles known as the sterno-tracheales (fig. 2). In the chicken they are well developed muscles which take origin, one on each side, from the antero-lateral process of the sternum. Their fibers are directed obliquely ventrally and cephalad, and reach

the trachea at the level of the tenth ring (fig. 2). Here, as beyond, both muscles are firmly bound to the trachea by a strong common fascial sheath. But despite their fascial attachment, none of their fibers is inserted at this level. Springing from the medial surface of both muscles are several small bundles of fibers which, directed obliquely cephalad, are inserted on the tracheal rings, from the thirteenth to the twenty-first. Cephalad to these muscular bundles both muscles, now much diminished in size, proceed along the sides of the trachea to their final insertion on the ventral cartilages of the upper larynx. The exact extent and manner of insertion of the sterno-trachealis muscles offers a field for further investigation. The action of these muscles will be discussed in the section on function.

Two pairs of short bundles of muscle fibers are found on the caudal end of the trachea. One pair (fig. 2) lies on the ventro-lateral aspect of the trachea, the other on its dorso-lateral aspect. Caudally these bundles end at the levels of the fifth to eighth rings. Cephalically the majority of their fibers enter, and apparently become a part of, the ventral and the dorsal margins of the sterno-tracheales respectively. The fibers which do not enter the sterno-trachealis muscles are inserted on the respective ventral and dorsal walls of the trachea. Some of the fibers of the ventro-lateral bundles spread out cephalad to the level of the twelfth ring, to cover the ventral surface of the trachea, and certain fibers from the dorso-lateral bundles also spread out to cover its dorsal surface.

*d. Sexual differences.* Sexual differences are very marked in song birds, especially as regards the size of the labia and the syringeal muscles. In male aquatic birds the tympanum is a large bony swelling projecting from the left side of the trachea, while in females the tympanum, though present, does not project beyond the tracheal walls. The main sexual difference to be noted in the syrinx of chickens is one of size (cf. figs. 5 and 6 for which males and females of the same varieties were chosen). Male birds are usually larger than females, and so some difference would naturally be expected in the size of their voice organs. But this difference in size of individuals is not sufficient

to account for all the differences observed. Quite naturally the sterno-tracheal muscles are noticeably smaller in females; but the bony rings above the tympanum, these, in the male (fig. 5) are entirely different in size and shape from the corresponding rings in the female (fig. 6). Again, in the male the tympanum is composed of the first four tracheal rings, while in the female only the first three form this structure. The tympanic membranes, however, exhibit no marked difference.

It is not an uncommon thing to hear a female chicken try to crow. The sounds she produces are distinct, and cannot be mistaken for anything other than an attempted crow. Judging from the structure of the syrinx, there is no apparent reason why the female should not be able to crow perfectly, provided the instinct for it were properly developed. Häcker ('00) calls attention to the fact that the females of certain species of song birds, when kept in captivity, learn to sing as charmingly as the males. Barrington (1773) gives numerous examples of one species being trained to sing the song of another species.

## *2. Microscopic anatomy*

For this phase of the investigation, cross, coronal, and longitudinal sections were made through the syrinx of both male and female adult chickens. The sections were cut five, ten, fifteen, and twenty micra in thickness. The following stains were used: iron hematoxylin, picrofuchsin, Mallory's aniline-blue connective tissue stain, Weigert's resorcin-fuchsin elastic tissue stain, Unna's orcein, and Mayer's mucicarmine.

In figure 5 all of the rings, the half-rings, the cartilages, and the pessulus are seen in cross section. It is to be noted that the tracheal rings cephalad to the second are completely ossified. Each consists of a thick peripheral layer of compact bone within which is a central area of cancellous bone. A distinct periosteum surrounds the whole. The cavities of the cancellous portions contain bone-marrow not unlike that found in other bones of birds.

As stated before, the first four tracheal rings are united to form the tympanum. The first two rings are composed of hya-



line cartilage throughout; the third and the fourth are entirely of bone. The remaining rings of the trachea are also bone, but each is separated from the next by a small space, which is bridged by a heavy band of fibrous tissue which, on reaching the rings, becomes continuous with their periosteum.

The first and the second intermediate syringeal cartilages are small, thin, cartilaginous bars, each having a very thin and indistinct perichondrium. These cartilages are very rudimentary in every respect. The and the third fourthin termediate cartilages are much better developed and possess well marked perichondria.

Each intercartilaginous space, from the caudal border of the tympanum to the cephalic border of the fourth intermediate cartilage, is occupied by a strong thick band of fibrous tissue. As determined, after staining with Weigert's elastic tissue stain, these bands are composed almost entirely of elastic fibers.

As seen in figure 5, the first and the second bronchial half-rings are cartilaginous, but as previously mentioned, the ventral extremity of the first becomes ossified in old individuals.

The pessulus appears triangular in cross section, and is ossified throughout its entire extent. The marrow cavity is usually quite extensive, and is surrounded by a comparatively thin layer of compact bone. The lateral surfaces of the pessulus, as well as its cephalic and lateral borders, are covered by the mucous membrane of the respiratory tract. A distinct areolar tunic, or submucosa, especially marked over the cephalic border, connects the respiratory membrane to the periosteum of the pessulus. A somewhat different arrangement is found on the caudal surface of the pessulus. Here an areolar coat, continuous with the bronchial submucosa, connects the periosteum of the pessulus with the membranous reduplications in the interbronchial interval immediately cephalad to the bronchidesmus.

Above the tympanum the epithelium of the tracheal mucous membrane is of the stratified ciliated columnar variety, with a distinct basement membrane. The tunica propria is well developed and contains many lymph cells as well as numerous distinct lymph nodules. These nodules, which are most numerous



in the region of the tracheal bifurcation, are quite near the surface, since they lie just beneath the epithelium. Where these lymph nodules occur the epithelial cells are non-ciliated.

A distinct submucosa is present throughout practically the entire extent of the mucous membrane of the trachea and the bronchi, and connects this mucosa to the interannular membranes and the periosteum, or the perichondrium of the tracheal rings, the intermediate syringeal cartilages, and the bronchial half-rings. There is no sharp line of demarcation between the submucosa and the tunica propria, but the former is easily distinguished from the latter in that it is less dense and contains fewer lymph cells. Over the tympanic membranes the tunica propria is thin and sparse and a submucosa is indistinguishable.

At the level of the caudal border of the third tracheal ring, the epithelial cells lose their cilia and immediately become columnar in shape. There is a gradual transition from stratified ciliated columnar epithelium to stratified squamous. The latter is made up of several layers at the lower border of the tympanum, but becomes thinner and thinner, as far as a point slightly caudad to the fourth intermediate syringeal cartilages, where it consists of only a single layer of flat cells, covering the internal, or medial, surfaces of the external tympanic membranes. The lateral surfaces of the internal tympanic membranes, or those surfaces of these membranes which face the lumen of each primary bronchus, are covered by a similar simple epithelium. Below the limits of the external tympanic membranes the epithelium gradually becomes thicker, and about midway between the first and second bronchial half-rings it again assumes the characteristics of a ciliated columnar epithelium.

The disposition of the squamous epithelium suggests that the portions of the syringeal walls covered by it are subject to considerable movement.

As stated above, the vibrating membranes are covered by a thin epithelium composed of a single layer of flattened cells; beneath this is the tunica propria which is thin and less distinct than that found in other parts of the respiratory tract. Beneath the tunica propria is a thin, dense layer of tissue which comprises

the vibrating membranes proper. This layer is composed of numerous white and elastic fibers. It is to be noted that there is a tendency for all layers to become much thinner as the tympanic membranes are approached (fig. 5).

The external and the internal labia, which occur in song birds in connection with the tympanic membranes, were not observed in the chicken.

The semilunar membrane is poorly developed in the chicken. It is nothing more than a modification of the mucosa over the cephalic border of the shaft of the pessulus. The epithelium in this position is thickened and belongs to the stratified ciliated columnar type. The submucosa too, as already mentioned, is especially thick. Thus it is evident that the semilunar membrane in the chicken is but a poor representative of this structure as found in many song birds.

## II. DEVELOPMENT

For this division of the present investigation the following selected stages were used.

### *Period of Incubation*

|           |           |           |
|-----------|-----------|-----------|
| 68 hours  | 152 hours | 272 hours |
| 70 hours  | 164 hours | 284 hours |
| 72 hours  | 176 hours | 296 hours |
| 74 hours  | 188 hours | 308 hours |
| 78 hours  | 200 hours | 320 hours |
| 80 hours  | 212 hours | 332 hours |
| 92 hours  | 226 hours | 356 hours |
| 104 hours | 236 hours | 380 hours |
| 128 hours | 248 hours | 404 hours |
| 140 hours | 260 hours | 452 hours |

Twenty-four hours after hatching.

Serial cross sections were made of nearly all the stages and serial coronal sections were made of a large number of the stages. In the early stages series were made of the entire embryo while later only the syringeal region was sectioned. For the most part the sections were cut ten micra in thickness. In the more important stages a drawing of the embryo was made before sectioning, which was utilized in obtaining the plane of sectioning

and in obtaining the curvature of the backline of the embryo for reconstructions. Most series were stained with iron or alum hematoxylin, with picrofuchsin or eosin as counter stains. Some of the later stages, however, were stained with Mallory's anilin-blue connective tissue stain, Weigert's resorcin-fuchsin elastic tissue stain, and Mayer's mucicarmin.

In transverse sections of a 68 hour embryo, the epithelial tube of the foregut is surrounded by a condensed mass of mesenchyma. This tube, which in its most cephalic part is cylindrical, when followed caudad, enlarges and becomes triangular in shape. Extending ventrolaterally from this tube are two beginning diverticula, the anlagen of the trachea and the bronchi. In length the right is 0.05 mm. while the left is 0.03 mm.

Embryos of 74, 78, and 80 hours show a lengthening of the trachea and the bronchi, but the former is still relatively much shorter than the latter. Wunderlich called attention to this condition in *Fringilla domestica* and mentioned the fact that it represents a stage which is found throughout life in certain reptiles. This might be regarded as a homology, but such homology is not probable. It would seem that a logical explanation of this condition might be found in a study of the position and the relations of the organs of this region. Since the neck as such has not yet developed it is obvious that the trachea springs from the digestive tube and bifurcates at once at a point not far from the future position of the lungs. As the neck lengthens, the oesophagus and the trachea must keep pace. The trachea, therefore, becomes drawn out. This elongation goes on to such an extent in the chicken that the trachea eventually becomes much longer than the bronchi.

A 128 hour embryo shows a marked increase in the length of the trachea and the bronchi, and in a 140 hour stage these structures have nearly doubled in length. The right bronchus courses laterally and gives off seven sac-like branches which later become the so-called pipes of the lungs.

Since the tracheal bifurcation is the region of especial interest in this paper, the general development of the trachea and the bronchi will not be traced further.

*1. Mucous membrane*

In the early stages described above, the walls of the trachea and the bronchi consist of an epithelial tube surrounded by a loose mesenchyma. In the 68 hour stage the epithelium of this tube may be said to belong to the stratified variety. The boundaries between the cells are not well defined, but two or three layers of nuclei can be observed. The cells lining the lumen are mostly columnar with their nuclei placed in the ends distal to the lumen. The basal cells are much shorter and more irregular in shape. Each bronchus presents a well defined lumen.

No noticeable differences occur in the structure of the tube in embryos of 72, 74, and 78 hours. In an 80 hour embryo, however, the beginning of the basement membrane is seen as a small differentiated line which extends around the outside of the epithelial tube. Immediately below its origin from the pharynx the tracheal tube has a lumen of oval outline. More caudally it elongates from side to side until immediately above the bifurcation it is little more than a transverse slit. At this level the dorsal epithelial wall becomes thicker, while the ventral wall appears to fold inward and to come into contact with the dorsal wall, thus dividing the one slit-like lumen into two. The lumina of the two bronchi and the trachea have a smaller diameter at 80 hours than at any of the earlier stages. The epithelial walls have not thickened at this stage, but the mass of mesenchyma surrounding the digestive and respiratory tubes has condensed.

In 128 and 140 hour embryos, the lumen of the tracheal tube is so small as to seem almost obliterated in some sections. The thickness of the dorsal wall is nearly double that of the ventral. About 0.1 mm. cephalad to the bifurcation, the tracheal tube becomes compressed dorso-ventrally, thus a slit similar to that described above is produced. It possesses, however, a somewhat larger lumen.

The lumen of each bronchus possesses a greater transverse diameter than that of the trachea, except in that region immediately above the bifurcation of the latter. The dorso-medial portion of each bronchial wall is much thicker than any other part.

This seems to be due to a greater number of cell layers rather than to the elongation of the cells, since four distinct layers of nuclei appear in the thickened part, while only 2 or 3 layers are present in the thinner portions of the wall. The same condition explains the thickening of the dorsal wall of the trachea. A very distinct basement membrane is present in these stages.

In 152 and 164 hour embryos the lumen of the trachea is still very small. At a point 0.24 mm. above the bifurcation it begins to enlarge, not only in the transverse diameter, but in the dorso-ventral as well. A cross section of the lumen and walls of the tube at this level presents a figure which is almost a perfect square, and is much larger than that described in previous stages. Of the walls composing the square, the dorsal is thickest, the ventral thinnest, while the lateral are intermediate in this respect. The dorsal wall possesses four layers of nuclei, the lateral walls three, and the ventral wall two layers.

Figure 12, from an embryo of 176 hours, shows the relative thickness of the epithelium in the region of the tracheal bifurcation. The epithelium is thickest just cephalad to the bifurcation, where not less than three or four distinct layers of nuclei are visible. At the level of, and slightly caudad to the bifurcation, the number of cells gradually diminishes, until only two layers of nuclei are present. The epithelium projecting into the tracheal lumen at the point of the bifurcation appears slightly thicker than that lining the medial bronchial walls with which it is continuous. At this stage, too, slight irregularities or evaginations become noticeable in the inner walls of the epithelial tube. These are due to encroachments upon the tube, from without, of growing mesenchymal condensations which lie immediately beneath and in direct contact with the basement membrane of the epithelium. Only two of these evaginations have developed to a stage worthy of mention. They are somewhat caudad to the tracheal bifurcation, one projecting into the lumen from the lateral wall of each primary bronchus. Caudad to the site of these evaginations the epithelium again thickens and for some distance three layers of nuclei can be distinguished.



In a stage 24 hours later (200 hours), as well as in a 212 hour embryo, the epithelium shows a noteworthy characteristic. The nuclei of the cells bordering upon the lumina have migrated from the basal to the free ends of the cells, thus giving rise to a very prominent superficial row of nuclei. Immediately beneath this the other nuclei show an irregular arrangement. Although this characteristic arrangement of nuclei is noticeable in the 176 hour stage, it does not become very striking until the present stage is reached. In this stage also a second evagination (fig. 13) appears in the lateral wall of each bronchus, just cephalad to the one described as present in the preceding stage. At 248 hours many of these evaginations may be observed in the walls of both the trachea and the bronchi. In the latter they are confined, however, to the lateral walls.

A cross section of a 332 hour embryo shows two or three layers of nuclei in the epithelium, except in the region of the future tympanic membranes, where a single layer occurs. The cells in these regions are cuboidal. Throughout the region of the bifurcation of the trachea, a considerable number of goblet cells, as well as numerous cells exhibiting a somewhat differentiated clear area in their free ends, occur in the epithelial layer nearest the lumen. Numerous cilia are present on the free borders of some cells.

In a 404 hour embryo a very distinct layer of nuclei appears at the very base of the epithelium. These are much smaller than those found nearer the lumen. As the regions of the future tympanic membranes are approached, all cells between the layer of small nuclei are left to cover the membranes. These cells differ from those described in the 332 hour embryo in being much less cuboidal, smaller and possessing much smaller nuclei.

The most noticeable differences between the last embryo and one of 452 hours are (1) the nuclei of the cells covering the tympanic membranes have become much more flattened and in appearance suggest the nuclei of endothelial cells; (2) in addition to individual goblet cells, small groups consisting of three or more secreting cells are found in the epithelium. Each of these groups is slightly invaginated, leaving a shallow pit connecting

with the lumen of the respiratory tract. Each cell appears light and presents a pore-like opening which communicates with the common pit. The nuclei of these secreting cells are situated nearer the basement membrane than those of the typical epithelial cells. These structures are not found in the epithelium, covering the tympanic membranes. In a chick 24 hours after hatching, such groups of secreting cells are much more numerous and show a higher degree of development in having become more invaginated. As determined by staining with mucicarmine it is evident that these structures are the anlagen of the simple mucous alveolar glands found in this region. Well developed cilia project from the free borders of the typical columnar cells, but they are not seen in the region of the tympanic membranes.

The vacuoles, which occur in such great abundance in the mucous membrane of the digestive tube in human embryos (Johnson '10), were observed in the respiratory tube of chick embryos, but they are neither constant or abundant. In a 280 hour embryo, such vacuoles are quite numerous in the epithelium lining the medial bronchial walls, but in all other stages they are practically absent.

## *2. Cartilaginous skeleton*

In an 80 hour embryo the epithelial tubes constituting the trachea, the bronchi, and the oesophagus are all closely surrounded throughout their entire extent by a common area of mesenchyma which, in immediate relation to them, is much denser than it is a little beyond them. An inspection of figures 12, 13, and 7 will make clear this arrangement of the mesenchyma.

In the 128 hour stage that portion of the condensed area of mesenchyma surrounding the respiratory tract has become separated from that surrounding the digestive tube. They now appear as two distinct areas, that surrounding the trachea being the smaller. The condensed mesenchyma is, of course, surrounded in turn by loose, unmodified mesenchymal tissue.

In a 152 hour embryo the mesenchymal condensation, common to the entire respiratory tract, becomes more prominent in the region of the tracheal bifurcation than in any other portion.

Along the dorsal surface in this region the condensation is no thicker than in the preceding stages, but it is much thicker lateral and ventral to the bifurcation (fig. 7). It also extends between the bronchi, just caudad to their union with the trachea. In a 164 hour embryo, at the points in the lateral bronchial walls where the above mentioned evaginations of the epithelium occur, the aggregated mesenchymal cells arrange themselves into very compact areas. These areas appear quite round in cross section. In the center of each area are several unmodified cells which are immediately surrounded by others of the same type concentrically placed. This arrangement of mesenchymal cells is the beginning of the anlagen of the first bronchial half-rings which, of all the skeletal elements, are the first to appear. This anlage extends from the ventro-lateral to the dorso-lateral parts of each bronchus, but appears best developed in its middle portion. These anlagen are more marked in a 176 hour embryo and are represented in figure 7, as two rounded and elongated masses projecting dorso-ventrally around the lateral walls of the epithelial tubes of the bronchi a little below the tracheal bifurcation. Twelve hours later the mesenchymal cells just cephalad to these anlagen for the first bronchial half-rings become arranged so as to form two other areas of aggregated cells similar to the ones just described. These areas are the anlagen of the fourth intermediate syringeal cartilages, which at this stage lie very near those of the first bronchial half-rings.

At 200 hours the first half-rings, and the fourth intermediate cartilages stand out much more prominently, since the mesenchyma immediately surrounding them is now less dense (fig. 13). The fourth intermediate syringeal cartilages differ from the first half-rings only in extending farther ventrally. The anlage of the pessulus appears very faintly in the mesenchyma between the bronchi, and slightly caudad to the tracheal bifurcation. It occupies the ventral three-fifths of the distance from the ventral to the dorsal walls of the respiratory tube in the region of its bifurcation. In structure it does not appear different from the above described anlagen, but it is much larger than either of them. A V-shaped mass of condensed mesenchyma extends from

the cephalic border of the pessulus to the point of union of the two bronchi.

In a 226 hour embryo, the cells in the center of the anlagen have no definite arrangement, but are larger and more oval in shape than those in the preceding stages. The central cells, in enlarging, have crowded the outer cells together, thus causing the individual outer cells to appear flattened and the whole peripheral area to appear narrower. The first half-rings and the fourth intermediate syringeal cartilages are now separated by a greater space than in the stages previously described. The anlage of an additional cartilage appear just cephalad to the developing fourth intermediate syringeal cartilages. Though small, as yet, these anlagen, the beginnings of the third intermediate syringeal cartilages, are fused with the cephalic extremity of the ventral pyramid of the pessulus. Dorsally these anlagen extend only slightly more than one-half of the distance to the dorsal extremity of the pessulus where they end unattached. These structures appeared in only one out of two embryos taken at this stage. These anlagen are shown in figures 8 and 9, which make clear the relation and the extent of these and other developing skeletal elements. The first bronchial half-rings extend around the lateral walls of the bronchi but do not reach the pessulus at either extremity. The fourth intermediate syringeal cartilages arch cephalad to some extent and are fused with the ventral pyramidal extremity of the pessulus. Their dorsal ends are unattached and lie somewhat lateral to the dorsal pyramid of the pessulus. The developing pessulus has gradually assumed the same form as in the adult, except the shaft, which at this stage is round in cross section, rather than triangular. At this stage, too, the mesenchyma along the entire ventral surface of the trachea has become more condensed. This condensation represents the primordium of the anlagen of the tracheal rings.

The 248 hour stage shows a marked advance in the development of the skeletal parts (fig. 14). All the tracheal rings are represented by circular (in cross section) condensations of mesenchyma which extend around the lumen, immediately external and in contact with the epithelial tube. The first and the second



intermediate cartilages are here represented by extremely small masses which lack the regular cell arrangement found in the others. Ventrally they fuse with the ventral pyramid of the pessulus. Immediately caudad to the first half-rings is a mesenchymal area, which is apparently condensing to form the anlage for the second half-ring. None of the other bronchial cartilages are represented at this stage.

At 260 hours the central cells of the anlagen for the first half-rings, the fourth intermediate syringeal cartilages, and the pessulus appear slightly more separated from each other. As would be expected from the order of their first appearance this is most marked in the anlagen for the first half-rings, where true embryonal cartilage is closely approached, and least marked in the pessulus. The first seven half-rings are represented at this stage.

All the cartilaginous and the bony skeletal elements of the respiratory tract are represented in an embryo of 284 hours. The pessulus, the first and the second half-rings, the tracheal rings (except the first and the second) and the fourth intermediate syringeal cartilages are composed of embryonal or primary cartilage. The perichondrium is represented by a thin layer of cells which are very much elongated and rather sparsely distributed. All skeletal elements, other than those above mentioned, are still in the mesenchymal stage. Figure 10, drawn from a wax reconstruction of the syrinx of a 284 hour chick, shows the extent, the size, the form, etc., of the cartilages, and their relation to the epithelial tube. All the intermediate syringeal cartilages are fused to the ventral pyramid of the pessulus. The fourth are much the largest of these and closely approach the first half-rings in size. They present a decided cephalic arching in the middle portion of their course, and each shows a marked enlargement, which ends freely, but in close relation to the middle third of the dorsal pyramid of the pessulus. The third, with a diameter about one-half as great as that of the fourth, and placed slightly cephalad to the latter, pass around the epithelial tube and present free dorsal extremities, which lie cephalad and lateral to the apex of the dorsal pyramid of the pessulus. The second are much smaller and take a course parallel to that of the



third. At this stage, the first are not fused with the ventral pyramid, but are continuous with a mass of condensed mesenchyma which may be said to be a part of the apex of the pyramid. They pass around the trachea and end freely on its dorso-lateral aspect.

The first two tracheal rings are very small and, like the first three intermediate cartilages, are composed entirely of condensed mesenchyma. At both their dorsal and their ventral extremities they present a marked caudal dipping. The second tracheal ring is, however, somewhat larger and more advanced in its development than the first. It should be noted, also, that the first four tracheal rings are not in direct contact with each other as in the adult; but are separated by spaces which are occupied by embryonal fibrous tissue. The remaining tracheal rings call for no special description, since they differ from those of the adult only in size, in being circular in cross section, and in being composed entirely of cartilage. The ventral and dorsal extremities, or pyramids, of the pessulus, are cartilaginous throughout and are similar in shape to these parts in the adult. The shaft, however, as stated above, is round in cross section.

Second to the pessulus in size are the first bronchial half-rings. These half-rings in their middle portions, arch markedly caudad. Their ventral extremities are in close contact with the base of the ventral pyramid of the pessulus, but fusion has not taken place. Dorsally they end unattached, caudad and lateral to the pessulus. The second half-rings, which are much smaller and immediately caudad to the first, are connected with the latter at their ventral extremities by means of a small mass of condensed mesenchyma. A wax reconstruction of the syrinx at this stage (28½ hours) shows that the lower end of the trachea exhibits a slight compression (fig. 10). In the region of the intermediate syringeal cartilages this compression is well marked.

In a 320 hour embryo, all the skeletal elements are represented by primary cartilage. The first and the second intermediate syringeal cartilages are the last to be transformed into true cartilages, and at this stage are the smallest of all the skeletal

parts. These cartilages often appear fused into a single mass for at least a part of their extent.

In a 452 hour embryo, the last of the series, most of the tracheal rings cephalad to the tenth present two prolongations of precartilage, one of which is directed cephalad, the other caudad. The second to the tenth rings, inclusive, are now more or less perfect squares when seen in cross section. The third, the fourth, and the fifth, however, are much larger than the others (fig. 16). The first is round and still quite small. The intermediate cartilages also present a different appearance; being somewhat compressed, they now appear oblong in cross section. All of these cartilages are relatively much larger in all developmental stages than in the adult.

In cross section, the shaft of the pessulus is becoming slightly triangular, but it still does not possess the distinctly triangular shape of that of the adult. The first bronchial half-rings are only slightly compressed. At their dorsal extremities there are indications of fusion with the pessulus, while at their ventral extremities the two structures are separated only by their perichondria.

Wunderlich found the syringeal skeleton of a 17 day chicken embryo to be composed entirely of cartilage. He states that these elements are cartilaginous in *Fringilla domestica* and *Anas boschas* at the time of hatching. Rathke states that at hatching ossification has begun in the syrinx of the domestic chicken. In this investigation, however, no bone was observed in the syrinx of *Gallus domesticus* at the time of hatching, nor in stages one day later.

### 3. *Submucosa*

In the earlier stages, the anlagen of the skeletal elements, except the pessulus, are in such close contact with the epithelial tube that they produce actual evaginations of the epithelium (fig. 13). In a 284 hour embryo this is not true of the third and the fourth intermediate syringeal cartilages, and of the first half-rings; here, even typical mesenchymal cells occupy the considerable space between the above structures and the epithelial tube (fig. 15). The

mesenchyma referred to is continuous with, and seems to have migrated inward from, that occupying the space between the cartilages. The same condition exists between the tube and all the cartilaginous elements in the region of the tracheal bifurcation in a 296 hour embryo. In a 332 hour embryo these intervening mesenchymal cells possess numerous protoplasmic processes which form a loose network and which, at the 356 hour stage, become smaller and resemble short connective tissue fibers. In these spaces between the developing cartilages and the epithelial tube small, but distinct, fibers appear in a 404 hour embryo. These are true connective tissue fibers since they stand out very prominently when treated with Mallory's connective tissue stain. At this stage, the tissue now forms a loose connection between the epithelium on the one hand and the cartilage and intercartilaginous structures on the other. It contains blood vessels and nerves and constitutes the submucosa. As in the adult, there is no sharp line of demarcation between the submucosa and the tunica propria.

#### 4. *Musculature*

In the 164 hour embryo areas of differentiated cells may be observed between the ribs. Similar aggregates of cells extend from the antero-lateral process of the sternum to the trachea, somewhat above its bifurcation. The cells composing these aggregates have large oval nuclei, each of which possesses a distinct nucleolus; and their cytoplasm, which has a strong affinity for eosin, appears to be drawn out into short strands. These structures are more prominent in a 176 hour embryo. Wunderlich states that the muscular system begins to differentiate on the tenth day in *Fringilla domestica* and on the twelfth day in *Anas boschas*.

In the 188 hour stage numerous cytoplasmic strands have united to form long bundles which consist of minute fibrils. These bundles are present throughout the entire extent of the developing muscles, from the sternum to the cephalic end of the trachea. The nuclei are oval and several seem to belong to each bundle of fibrils. At the point where these bundles, or the

sterno-tracheal muscles, reach the trachea small additional masses of developing muscular tissue extend caudad along the dorso-lateral and ventro-lateral aspects of the trachea. These small additional masses seem to arise from, and certainly do not differ from, the tissue found in the developing sterno-tracheal muscles.

In the succeeding stages the muscles continue to develop by the addition of more cytoplasmic bundles. The nuclei of the muscle cells are easily distinguished from those of the developing connective tissue cells, which are present between the muscular bundles, for the former are more elongated and regular in shape. No other structural changes call for special mention until the 296th hour is reached, when faint cross striations appear in certain fibers. These, however, are quite rare.

At 452 hours the fibers have arranged themselves into more definite bundles. The fibers of these bundles show distinct cross striations, and numerous elongated nuclei appear to lie on or between them. Certain of these fibers are attached directly to the perichondrium of the lower tracheal rings.

##### *5. Intercartilaginous membranes*

When the aggregated mesenchymal cells arrange themselves to form the anlagen of the skeletal elements, small spaces exist between these anlagen. These spaces, also, are occupied by typical mesenchymal cells, but here they are fewer and farther apart (fig. 13). This condition is particularly well marked in a 248 hour embryo, in which it occurs between all of the developing cartilages except the first three intermediate syringeal cartilages. The widest spaces are those between the first half-rings and the fourth intermediate syringeal cartilages. In these spaces the mesenchyma shows a slight condensation, suggesting the anlagen of the external tympanic membranes. In a 260 hour embryo the cells lying near the periphery of this developing membrane have begun to elongate. Differentiation becomes more marked in each of the succeeding stages (272, 284, 296, 308, and 320 hours), but it is not until the 332 hour stage that small connective tissue fibers can be disclosed by the use of Mallory's stain.

Extending caudad from the lateral borders of the shaft of the pessulus in the 404 hour stage, and taking part in the formation of the medial bronchial walls, a well marked strand of connective tissue fibers is seen. A large number of these fibers become continuous with, and enter into the formation of, the bronchidesmus, which now stretches between the medial walls of the two primary bronchi.

On comparing figures 5 and 16 it will be seen that the tympanic membranes have not as yet reached a very high stage of development. From their structure and their size one would expect them to be quite inefficient as vibrating membranes, and capable of producing only very simple sounds. The simple sounds actually produced by the young chick, seem to verify this deduction.

At the time of hatching the various structures described are not essentially different, except for size, from those of the last stage (404 hours) discussed. It thus appears evident that the syrinx is very immature at the time of hatching. Between this time and the adult stage, the four most caudal tracheal rings unite, in the manner previously described, to form the tympanum; the pessulus and the tracheal rings cephalad to the second become ossified; and finally, the tympanic membranes become more like those of the adult. In addition to becoming thinner, these membranes become more extensive and their corresponding fibers become more longitudinally arranged.

#### *6. Summary of development*

The first indication of the respiratory tract was observed in a 68 hour embryo in which the laryngeal-tracheal groove and the developing bronchi were present. The trachea is at first very short and, like the bronchi, is composed of an epithelial tube and a loose surrounding mesenchyma. The bronchi are relatively much longer than the trachea in the beginning. Owing, however, to the rapid development of the neck, the trachea lengthens very rapidly and is much longer than either of the bronchi in a 128 hour embryo. The epithelium is stratified and, at 80 hours, rests upon a well defined basement membrane.



Immediately above the bifurcation, the lumen of the tube is at first slit-like in shape, but in later stages, 152 and 164 hours, it is almost square in cross section. Its dorsal wall is about two times as thick as its ventral wall.

The developing cartilages produce slight evaginations of the epithelium of the respiratory tube. In a 248 hour embryo these evaginations occur along the entire length of the trachea and the bronchi.

At 332 hours only a single layer of cuboidal epithelial cells covers the future tympanic membranes, while immediately cephalad and caudad to these membranes two or three layers of nuclei can be distinguished. A distinct layer of small nuclei appears at the base of the epithelium. In the tympanic regions this is the only layer of nuclei present. The cells possessing them are slightly cuboidal, but at 452 hours they become very flat.

Certain cells resembling goblet cells, and other cells with clear cytoplasm, are present in the 332 hour embryo in all portions of the epithelium, except that covering the tympanic membranes. They are quite numerous in the semilunar membrane. At 452 hours certain groups of secreting cells are found which, when stained with mucicarmine, and when traced through succeeding stages, were determined to be simple mucous alveolar glands.

Owing to the thickness of the sections it was difficult to detect the first appearance of cilia, but one can be reasonably sure of their presence in 248 and 260 hour embryos. They are very abundant in and following the 332 hour stage.

Vacuoles in the epithelium appeared in the 284 hour stage, but they were not very numerous in this embryo and are practically wanting in all other stages.

The skeletal elements are first represented by a condensation of the mesenchyma, which in a 152 hour embryo is most marked ventrally in the region of the bifurcation. The anlagen of the first bronchial half-rings appear just caudad to the bifurcation in the lateral walls of the bronchi, and are the only skeletal elements represented in the 164 hour stage. The next anlagen to appear are those of the fourth intermediate syringeal cartilages and of the pessulus which become detectable in 184 and 200 hour

embryos respectively. The third intermediate syringeal cartilages are represented at 226 hours. All of the tracheal rings, the intermediate cartilages, and the first two half-rings are represented in the 248 hour embryo.

Cartilage cells were first observed in the 284 hour embryo, in the first half-rings, in the fourth intermediate cartilages, and in the pessulus. The first tracheal ring and the first and the second intermediate syringeal cartilages are the last to differentiate. Differentiation for these cartilages begins in the 320 hour embryo. No bone has developed at the time of hatching, nor has the tympanum been fully formed.

The submucosa begins to develop at 284 hours. At first it is represented by mesenchymal cells, which later produce white and elastic fibers. These fibers, however, do not take Mallory's connective tissue stain until the 404 hour stage. At this time the submucosa contains numerous nerves and blood vessels.

The two sterno-tracheal muscles are quite well differentiated at 176 hours. At 188 hours the long cytoplasmic strands or processes of the developing cells are collected into bundle-like masses. Faint cross striations were first observed in a 296 hour stage. At 452 hours the muscles are not essentially different in structure from those of the adult.

The intercartilaginous membranes in early stages are not essentially different in structure from the submucosa. In a 260 hour embryo, the cells nearest their external surfaces elongate and later develop strands of connective tissue fibers which extend between the cartilages and attach to these membranes. Prominent bundles of such fibers extend caudally from the pessulus and enter into the formation of the medial walls of the bronchi, in a 404 hour embryo.

In early stages the external and the internal tympanic membranes do not differ in structure from the smaller intercartilaginous membranes of the trachea and the bronchi. At the time of hatching the tympanic membranes are quite thick. It is not until after this time that they become thinner and appear as true vibrating membranes.

## EXPERIMENTS ON FUNCTION

This division of the present paper may seem almost unnecessary since Duvernoy, Girardi, Cuvier, and others of the older investigators conclusively demonstrated that voice is produced in the lower larynx. Kitchner ('85), and other observers, however, have expressed their doubts concerning the correctness of the conclusions arrived at by these early investigators. Because of such doubts it was thought best to include these experiments.

*Experiment I.* The trachea of an adult cock was divided at its middle, after which the bird was set free with others. After the operation crowing occurred quite frequently, but the voice was somewhat modified. In order to study these modifications more carefully, phonographic records were made of the crowing before and after cutting the trachea. These records were made in the following manner. A normal adult cock was placed in a small room. After becoming accustomed to the new environment he had periods of crowing which were quite regular. An Edison phonograph was arranged just outside, so that the horn projected into the room through a small opening. This allowed the experimenter to operate the machine unseen. The early morning hours were found best for making records, as crowing was more frequent and regular at that time.

It is interesting to note that such birds usually crow about five or six times at intervals varying from ten to fifty seconds, then after an interval of fifteen minutes to one hour, they again begin to crow.

After a sufficient number of records had been made, the bird was deeply anaesthetised and a small part of the trachea exposed (fig. 17). This caused no noticeable difference in the voice. After sufficient recovery the trachea was treated with a local anaesthetic and cut entirely across. The cephalic end was tied tightly so there was no possible chance for air to pass from it through the upper larynx and the mouth. The caudal end was left open and allowed to protrude through the skin of the neck (fig. 18). The operation had no marked effect on the well-being of the bird, for, not more than two hours later this same cock,

when placed with some other chickens, was eating and crowing as though nothing had happened. Records of the crowing were made on the following morning. These records were preserved and carefully compared with those taken before the operation. This comparison showed that after the trachea had been cut, tones were produced as before but the pitch was noticeably higher. It must be admitted, therefore, that the trachea and upper larynx serve to modulate voice, just as the pharynx and cavities in the mouth serve the same function in mammals.

Similar experiments were performed on the domestic duck with precisely the same results.

Through the kindness of Dr. Max Meyer, professor in experimental psychology, University of Missouri, the author is able to give here the exact changes in pitch which result from dividing the trachea. Dr. Meyer determined the pitch of the voice of a normal adult male. In this particular individual he found the normal tone was one of about 375 double vibrations per second. The pitch is constant from time to time. It is interesting to note that there is also but little difference in the pitch from the beginning to the end of crowing. It was observed, however, that the pitch is slightly higher at the beginning, there being a fall of not more than twelve double vibrations.

After the trachea was divided the number of double vibrations was increased to about 500 per second.

The voice in crowing is not interrupted, but is produced by one continuous flow of air causing vibrations of the tympanic membranes. Ordinarily a single crow lasts for about three or four seconds. But in one individual it was observed that after the trachea was divided crowing extended over scarcely a single second of time. As explained by Dr. Meyer, there are two possible reasons for the time of crowing being so much reduced. First, when the neck is stretched pain may result from the wound. Second, the tone produced is so unnatural that it is soon discontinued.

*Experiment II.* Since the lungs of birds are not elastic structures as in mammals, but are more solid, it is obvious that other organs with a large air capacity must be present, and further,

that such organs must be capable of exhaling air in greater or less amount at the will of the bird. Such structures are found in the air sacs which are present throughout the thoracic and abdominal regions and are continuous with the cavities of neighboring bones.

A chicken was anaesthetized, the humerus sawed through at its middle and a tightly fitting piece of rubber tubing placed over the central stump. When air was forced through the tube into the air sacs, by way of the humerus, sound was produced. This varied in pitch with the amount of air pressure used, greater pressure resulting in higher pitch. The trachea was then divided, but this produced no noticeable result, since the bird was unable to control the length of the trachea or the width of the glottis by muscular contraction. Finally, the cervical air sacs were punctured after which it was impossible to produce voice in this artificial manner, thus proving Hérisaout's statement that when these sacs were ruptured birds are unable to sing. He explains this inability to sing as follows: Since the air sacs form air cavities around the syrinx, it can be seen that there is a tendency to equalize pressure on each side of the tympanic membranes, but when sudden gusts of air are forced out through the bronchi the equilibrium is disturbed. Thus the membranes are set into vibration. It is evident that all structures through which the voice passes from the syrinx to the exterior act as resonators. So far as size and extent are concerned, the trachea forms a large part of this resonating system.

*Experiment III.* In that part of this paper dealing with structure, it was noted that the sterno-trachealis muscles extend along the sides of the trachea from the twelfth ring to the upper larynx. When a pair of electrodes were applied to this muscle in an anaesthetized chicken, it was seen by its contraction to shorten the trachea from one-fourth to one-third of its original length. It should be recalled that the typical tracheal rings are so arranged that they may overlap when the muscles contract. The trachea may thus act as a pipe or horn capable of being lengthened and shortened. This has a direct influence upon pitch as was shown by the following experiment: The so-called thoracic cavity was



opened and one of the sterno-tracheal muscles was divided at A (fig. 2). After this operation the tones produced by the chicken were of a somewhat different quality. When both muscles were cut the quality of voice was even more distinctly modified. Obviously the division of these muscles was responsible for the changes produced. Some weeks later this same chicken was anaesthetized and electrodes applied both directly to the sterno-tracheales and to the nerves supplying them. It was observed, with some surprise, that the division of these muscles apparently had but little effect upon the actual shortening of the trachea. On stimulation the muscles immediately contracted and produced a marked shortening. By way of explanation, it should be recalled that the tympanum is attached to the pessulus and the first intermediate syringeal cartilages only by elastic membranes; that, although the ventral ends of the intermediate cartilages are attached to the ventral pyramid of the pessulus, the dorsal ends of these cartilages are unattached, and that all of these cartilages are connected with each other by the intercartilaginous membranes. Further, the external tympanic membranes are attached to the caudal borders of the fourth intermediate cartilages. Now, if the tympanum be drawn cephalad it is evident that the external tympanic membranes will, indirectly, be made more tense. Under normal conditions stimulation of the sterno-tracheales serves to shorten the trachea. They tend to pull the caudal end of the trachea cephalad; especially is this the case if there be a simultaneous contraction of the dorso-lateral and ventro-lateral muscular bundles previously described. The extent of this shortening of the trachea is prevented, however, to some extent by the sternal attachments of the sterno-tracheales. Hence, when these attachments are severed, the contraction of the tracheal parts of these muscles tend to make the external tympanic membranes more tense and a modification in the quality of the voice is the result. It is of course probable that other factors, more physical than the above, also share in the production of the modification observed.

From their structure it is evident that the external and the internal tympanic membranes are vibrating structures. As in

mammals the stratified ciliated columnar epithelium of the larynx is transformed into the stratified squamous layer over the true vocal cords, and submucous glands are absent, so in case of the syrinx of the chicken the stratified ciliated columnar epithelium is changed into a squamous epithelium over the tympanic membranes. This squamous epithelium, however, is composed of but a single layer. The prominent glands of the mucosa are also absent from these membranes.

The semilunar membrane was believed by Savart, Wunderlich, and others, to play an important part as a vibrating structure. It is said to be more prominent in songsters than in other birds, but Häcker ('00) pointed out that in black birds its epithelium is of the stratified ciliated columnar type, and that it probably is not of much importance in the production of voice, and also that it is often as well developed in songless species as in the best songsters. This structure certainly does not act as a vibrating membrane in the chicken for it is covered with stratified ciliated columnar epithelium, and mucous glands are just as abundant in it as in any unmodified part of the epithelium.

## CONCLUSIONS

### STRUCTURE

1. The syrinx of the domestic chicken belongs to the tracheo-bronchialis type, and is quite simple when compared with the voice organ of song birds.

2. No intrinsic muscles are present in the syrinx of *Gallus domesticus*. The extrinsic paired sternotrachealis with its caudal prolongations constitute the entire musculature of the syrinx.

3. The rigid skeleton is very highly modified. The first four tracheal rings are imperfectly fused to form the tympanum. The four intermediate syringeal cartilages are continuous ventrally with the ventral pyramid of the pessulus, while dorsally they end unattached. The first bronchial half-rings are large and in adults are attached and fused at both ends to the pessulus. The pessulus is the largest of all skeletal parts and lies dorso-ventrally at the junction of the bronchi, in a plane transverse to the long

axis of the trachea. The tracheal rings, the pessulus, and the ventral ends of the first half-rings become ossified, while all other skeletal parts remain cartilaginous.

4. The external tympanic membranes appear between the fourth intermediate syringeal cartilages and the first half-rings, while the internal tympanic membranes extend from the caudal borders of the pessulus to the bronchidesmus and represent merely a modified part of the medial bronchial walls.

5. The syrinx is lined with stratified ciliated columnar epithelium containing numerous simple alveolar glands. Upon approaching the tympanic membranes this columnar epithelium is transformed into a stratified squamous epithelium which becomes a single layer of flattened cells over the membranes proper.

6. The tympanum is attached to the remainder of the syrinx only by elastic membranes.

#### DEVELOPMENT

1. The first indication of the respiratory system was observed in a 68 hour embryo in which the laryngeotracheal groove and the bronchi were represented. At first the trachea is much shorter than the bronchi, but with the development of the neck, it becomes, after the 140 hour stage, relatively much longer than the bronchi. The walls of the trachea and the bronchi are at first composed only of epithelium which contains two or three rows of nuclei.

2. The mesenchymal condensation common to the entire epithelial tube first becomes markedly prominent at the tracheal bifurcation in an embryo of 152 hours.

3. The anlagen of the first bronchial half-rings appear in a 176 hour embryo, those of the fourth intermediate syringeal cartilages appear 12 hours later. The anlagen of the third intermediate syringeal cartilages and the anlage of the pessulus are present at 200 hours.

4. Distinct cartilage cells were first observed in the first bronchial half-rings.

5. The first four tracheal rings have not united to form the tympanum at hatching, nor have the other skeletal elements

taken the shape of those found in the adult. No bone is present at the time of hatching.

6. Ciliated cells are present in stages beyond 248 hours but were not observed in the region of the future tympanic membranes.

7. Mucous cells were first observed in 332 hour embryos and only in later stages were they found arranged in the form of simple alveolar glands.

8. Muscular tissue is differentiated in the 176 hour stage. Muscle fibers showing faint cross striations appear at 296 hours. At 452 hours the muscles are well developed.

9. At the time of hatching the tympanic membranes are thick. They are covered, however, as in the adult, with a single layer of epithelial cells.

#### FUNCTION.

1. That the syrinx is the true voice organ of the chicken is evident from the following deductions:

First, structurally it is the only part of the respiratory tract capable of producing sound.

Second, when the trachea is divided and the cephalic portion tightly tied, the chicken is still able to crow.

Third, after division of the trachea, voice can be reproduced artificially by forcing air into the air sacs.

2. The upper larynx serves only to modulate the voice.

3. The sterno-tracheal muscles, by their contraction, shorten the trachea and modify pitch. They also draw the tympanum cephalad, thus indirectly varying the tenseness of the tympanic membranes.

4. The air sacs are necessary in voice production, for voice could not be produced artificially after puncturing the cervical sacs.

## PLATE 1

### EXPLANATION OF FIGURES

- 1 Dissection of ventral portion of neck and cephalic portion of thorax to show course of trachea,  $\times \frac{1}{3}$ . Adult male.
- 2 Dissection of syringeal region. The ventral body wall and the heart have been removed to show the syrinx in position,  $\times 3$ . Adult male.
- 3 Pessulus,  $\times 16\frac{1}{2}$ . Young adult male.

### ABBREVIATIONS

|   |  |
|---|--|
| <i>a</i> , apices of pyramids   | <i>ep.</i> , epithelium  |
| <i>an</i> , <i>BI</i> , <i>BII</i> , anlage of first and second bronchial half-rings                          | <i>infl.</i> , lower larynx or syrinx                                    |
| <i>an.i.s.c.I, II, III, IV</i> , anlagen of first, second, third and fourth intermediate syringeal cartilages | <i>i.m.</i> , interannular membranes                                     |
| <i>an.pes.</i> , anlage of pessulus   | <i>i.t.m.</i> , internal tympanic membrane                               |
| <i>ant.l.p.</i> , antero-lateral process of sternum   | <i>i.s.c.</i> , intermediate syringeal cartilages                        |
| <i>br.</i> , bronchidesmus  | <i>i.c.m.</i> , developing interannular and intercartilaginous membranes |
| <i>b.v.</i> , blood vessels   | <i>lat.b.</i> , lateral borders of pyramids                              |
| <i>b.w.</i> , body wall   | <i>l.b.</i> , lateral border of shaft                                    |
| <i>B.</i> , typical bronchial half-rings  | <i>m.c.</i> , marrow cavity  |
| <i>b.</i> , ossified portion of shaft   | <i>m.b.w.</i> , medial bronchial wall                                    |
| <i>b.sur.</i> , basal surface of shaft and pyramids   | <i>mes.</i> , mesenchyma   |
| <i>BI, BII, BIII</i> , first, second and third bronchial half-rings   | <i>oe.</i> , oesophagus  |
| <i>b.m.</i> , basement membrane   | <i>pes.</i> , pessulus   |
| <i>c.</i> , crop  | <i>per.</i> , developing perichondria                                    |
| <i>cor.</i> , coracoid  | <i>st.h.m.</i> , sterno hyoid muscle                                     |
| <i>c.d.m.</i> , ventro lateral muscle bundles   | <i>sup.l.</i> , upper larynx   |
| <i>c.mes.</i> , condensed mesenchyma  | <i>st.tr.m.</i> , sterno trachealis muscle                               |
| <i>d.sur.</i> , dorsal surface of pyramid   | <i>s.</i> , shaft of pessulus  |
| <i>dor.p.</i> , dorsal pyramid of pessulus  | <i>sem.m.</i> , semilunar membrane                                       |
| <i>e.t.m.</i> , external tympanic membrane  | <i>sm.</i> , submucosa   |
| <i>ep.t.</i> , epithelial tube  | <i>tr.</i> , tracheal rings  |
|   | <i>T.</i> , tympanum   |
|   | <i>v.c.</i> , vertebral column   |
|   | <i>ven.p.</i> , ventral pyramid  |
|   | <i>ven.s.p.</i> , ventral surface of pyramid                             |



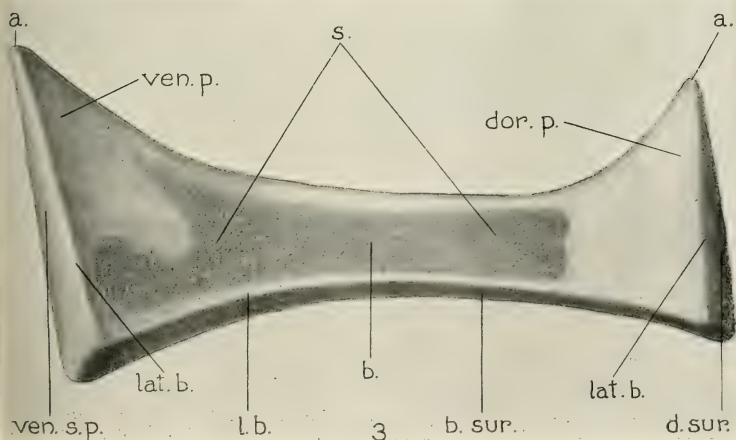
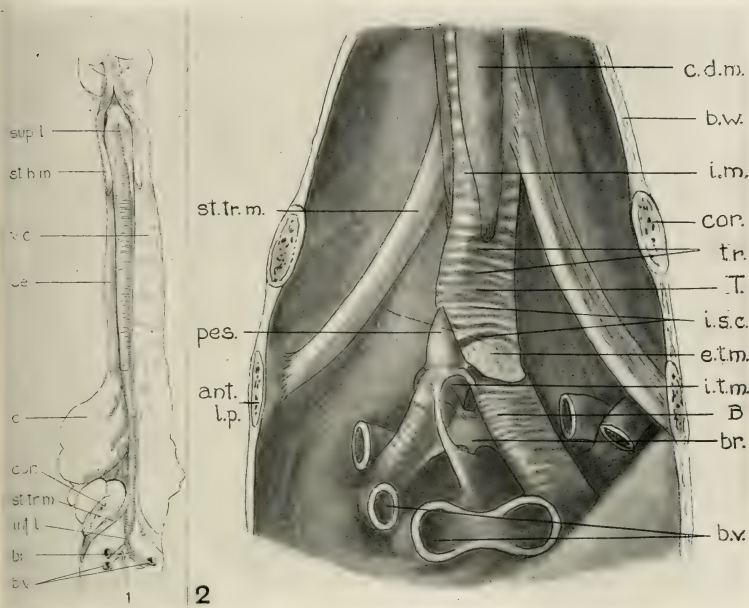
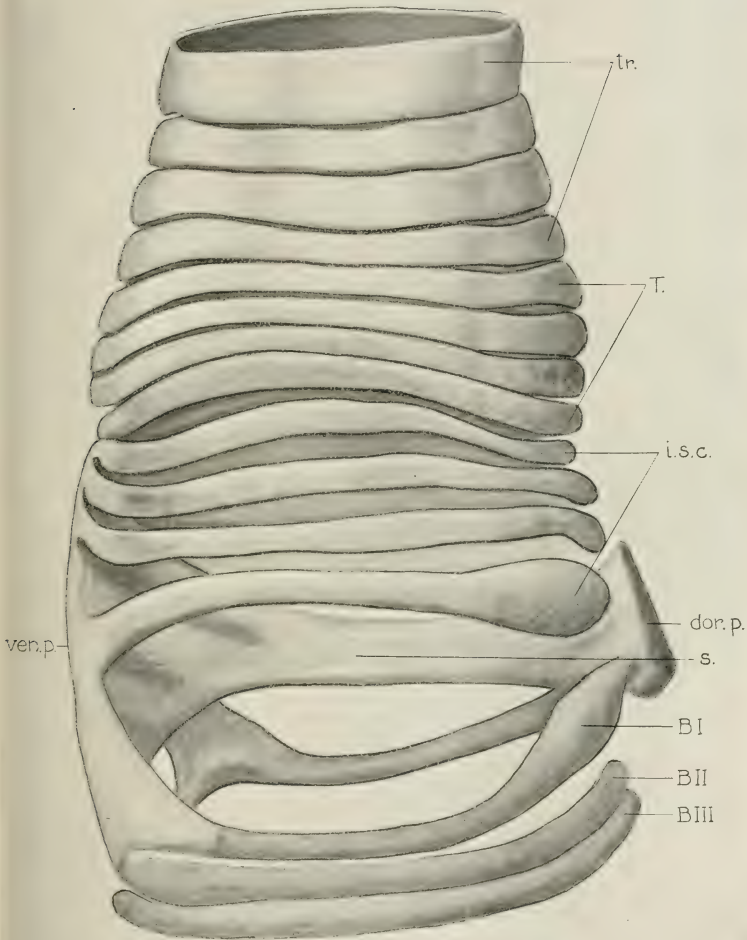


PLATE 2

EXPLANATION OF FIGURES

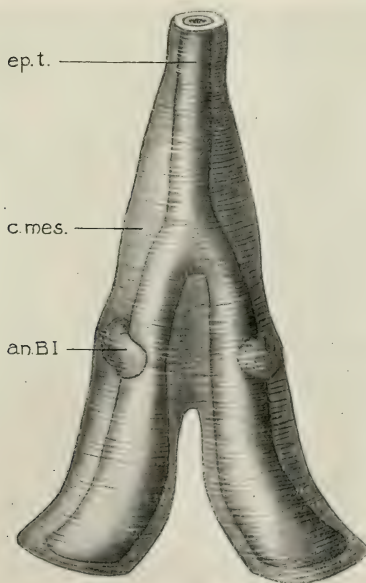
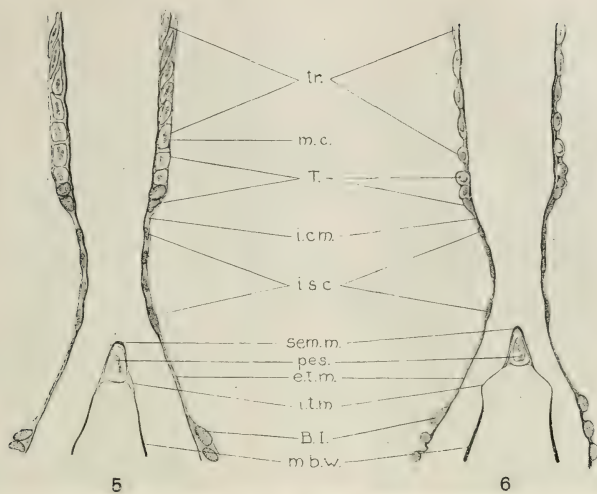
- 4 Wax reconstruction of syringeal skeleton,  $\times 8$ . Adult male.



### PLATE 3

#### EXPLANATION OF FIGURES

- 5 Mid-coronal section of syrinx,  $\times 8\frac{1}{2}$ . Adult male.
- 6 Mid-coronal section of syrinx,  $\times 8\frac{1}{2}$ . Adult female.
- 7 Transparent drawing of wax reconstruction of epithelial tube and condensed mesenchyma in region of tracheal bifurcation. Ventral aspect,  $\times 50$ . 176 hour chick embryo.





## PLATE 4

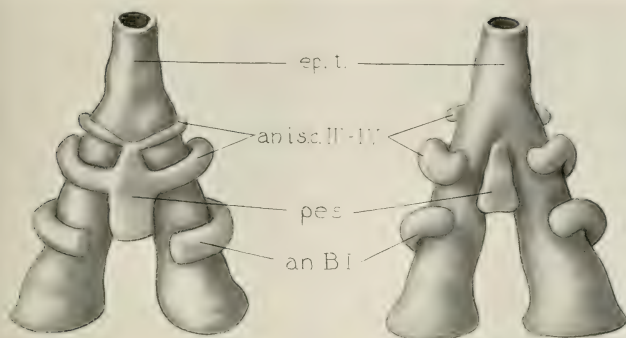
### EXPLANATION OF FIGURES

8 Wax reconstruction of epithelial tube and related developing skeletal structures in the region of the tracheal bifurcation. Ventral aspect,  $\times 50$ . 226 hour chick embryo.

9 Same as 8. Dorsal aspect,  $\times 50$ .

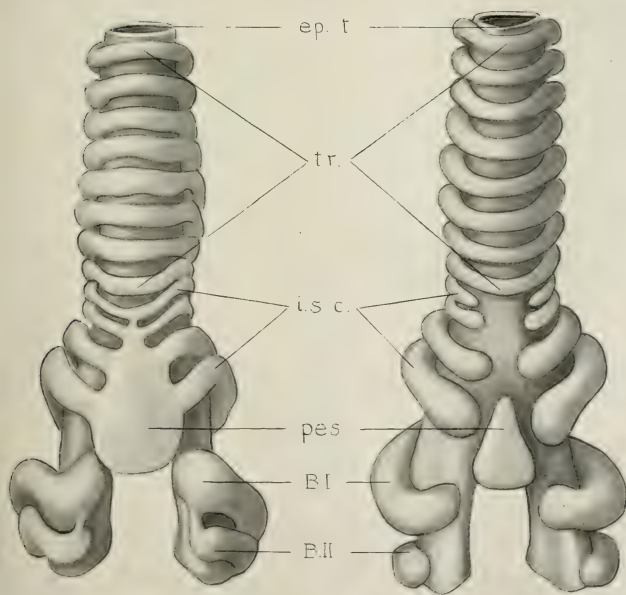
10 Wax reconstruction of epithelial tube and related skeletal elements. Ventral aspect,  $\times 50$ . 284 hour chick embryo.

11 Same as 10. Dorsal aspect,  $\times 50$ .



8

9



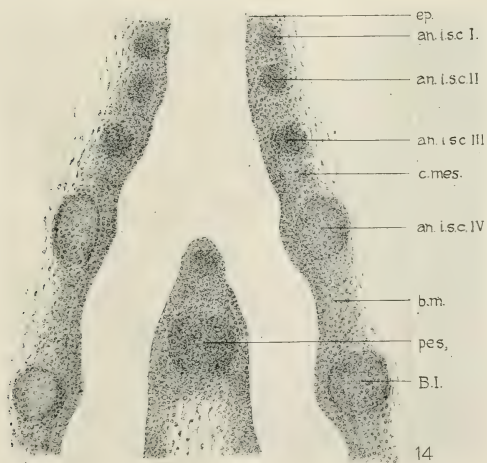
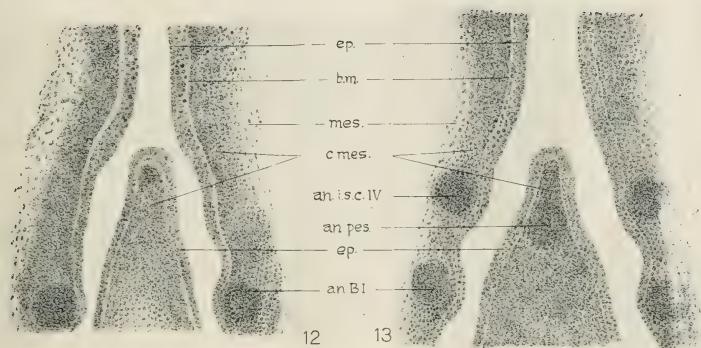
10

11

## PLATE 5

### EXPLANATION OF FIGURES

- 12 Coronal section of the trachea in the region of its bifurcation,  $\times 176$ .  
176 hour chick embryo.
- 13 Coronal section of the trachea in the region of its bifurcation,  $\times 100$ .  
200 hour chick embryo.
- 14 Coronal section of the trachea in the region of its bifurcation,  $\times 104$ .  
248 hour chick embryo.



## PLATE 6

### EXPLANATION OF FIGURES

15 Coronal section of the trachea in the region of its bifurcation,  $\times 64$ .  
284 hour chick embryo.

16 Coronal section of the trachea in the region of its bifurcation,  $\times 25$ .  
452 hour chick embryo.



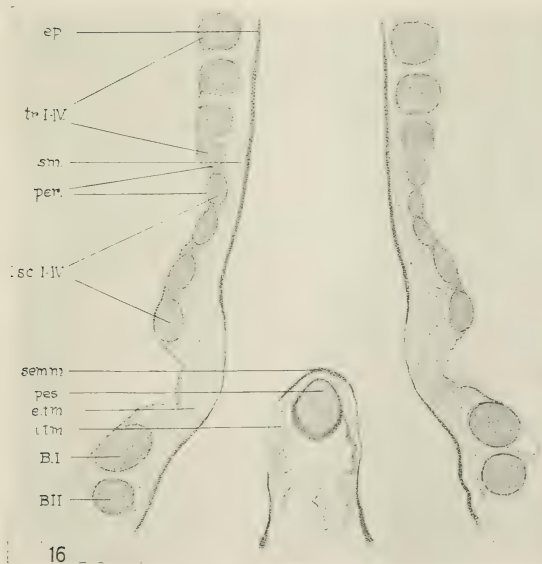
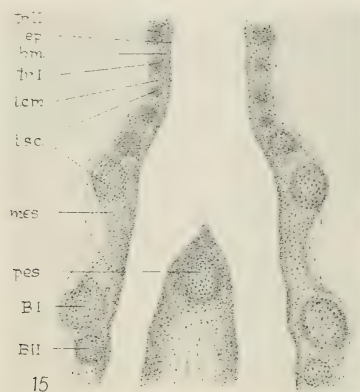


PLATE 7

EXPLANATION OF FIGURES

- 17 Photograph, after exposure of the trachea.
- 18 Photograph after division of the trachea.





# STUDIES ON CILIATED CELLS

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ONE TEXT FIGURE AND FOUR PLATES

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## I. INTRODUCTION

Since ciliary movement was first noticed by Anton de Heide, in 1683, ciliated cells have been a problem for various investigators. Their structures were studied by Friedreich ('58), Marchi ('66), Eberth ('66), Engelmann ('68, '79, '80), Eimer ('77), Frenzel ('86) and others; above all Engelmann and Frenzel gave detailed accounts of the matter, so there has been left but little to add as regards their gross structure. According to these authors, ciliated cells are provided, on their distal borders, with rod-like corpuscles, named basal knobs (Engelmann's 'Fussstückchen') or basal rods (Frenzel's 'Fussstäbchen'), with which the cilia are connected. These corpuscles—later termed basal corpuscles by Apáthy ('97)—were frequently recognized by several investigators and regarded as a constituent of great importance of the ciliary apparatus, which consists of cilia, basal corpuscles and rootlets.

The question also, whether the factor which produces the ciliary movement is to be sought in either of these three structures or in any other structure; especially the question as to the significance and development of basal corpuscles, were frequently discussed, and have not yet been decided definitely. Nearly about the same time ('98), but independently of each other, Henneguy and Lenhossék announced their opinion as to these questions. They regard basal corpuscles as derived from central corpuscles and as representing a kinetic centre of the ciliary movement, and point out, as evidence in support of this view, the morphological, topographical and chemical resemblance between these two structures, and the behavior of central corpuscles or similar bodies to the tails of spermatids of certain invertebrates and to those of antherozoids of certain plants. Whether or not this hypothesis (Henneguy-Lenhossék's hypothesis) expresses the truth, can be solved, I think, only by studies of the question of the existence or non-existence of centrosomes and of mitosis in ciliated cells, and especially by that of ciliogenesis. Benda ('00), Fuchs ('04), Ikeda ('06) and others believe they have found that the basal corpuscles take origin from the

dividing centrosome, whereas Gurwitsch ('01) and Wallengren ('05) assert, from their studies of the genesis of the cilia, both embryologically and after mitosis of ciliated cells, that the basal corpuscles are not derived from the centrosome, but differentiate in the cuticle or superficial layer of the cell-plasm, and that, accordingly, there is no reason to accept the Henneguy-Lenhossék's hypothesis.

In the present paper I intend to give an account of the structure of ciliated cells, their de- and regeneration, and the development of cilia, an account, which would throw some light on the problem above mentioned.

## II. MATERIALS AND METHODS OF INVESTIGATIONS

An extensive comparative study being necessary, I have fixed epithelia from various regions of invertebrates and vertebrates, which may be tabulated as follows:

### *Invertebrates*

|                   |            |
|-------------------|------------|
| Intestine.....    | { Anodonta |
|                   | { Limax    |
|                   | { Helix    |
| Gill } .....      | Anodonta   |
| Foot } .....      |            |
| Hepatic duct..... | Helix      |

### *Vertebrates*

|                           |   |                 |
|---------------------------|---|-----------------|
| Oviduct                   | { | Rana temporaria |
| Pharynx                   |   | Rana esculenta  |
| Gall-duct                 |   | Bufo            |
| Efferent tubule of testis |   | Triton          |
| Epididymis                | { | Trigonocephalus |
| Gall-duct                 |   | Eliaphis        |
| Efferent tubule of testis |   | Lizard          |
| Trachea                   |   | Mouse           |
| Efferent tubule of testis | { | Rat             |
| Epididymis                |   |                 |

### *Embryonic materials*

|                       |   |                        |
|-----------------------|---|------------------------|
| Ciliated infundibulum | { | Hyla, larvae           |
| Tela chorioidea       |   | Rhacophorus, larvae    |
| Pharynx               |   | Hynobius, larvae       |
| Epidermis             |   | Rana esculenta, larvae |

Small pieces of the materials were preserved either in Meves's fluid or in sublimate-acetic mixture, the result of which was in most cases favorable for the study of minute structures. With the former fluid it was passed through grades of alcohol, after washing thoroughly in running water, to 96 per cent alcohol; with the latter it was brought immediately into 70 per cent alcohol with iodine and then through grades of alcohol. Imbedded in paraffine through chloroform.

The sections were, for the most part, cut  $4\mu$  thick and stained on the slide. For staining I have mainly employed Heidenhain's iron-haematoxylin method, with or without counterstaining with plasma-dyes.

### III. STRUCTURE OF CILIATED CELLS

#### A. DESCRIPTIONS OF MY OWN OBSERVATIONS

1. *The foot of Anodonta.* The epithelium consists of a row of columnar ciliated cells (figs. 1, 2), the distal ends of which are bordered with a well-defined, relatively broad cuticle which stains faintly with iron-haematoxylin. In profile it is noticed that the cuticle is striated longitudinally, each stria appearing to be continuous with a cilium, which is from six to seven times as long as the height of the cuticle. Below the cuticle is a narrow zone, consisting of a series of basal corpuscles, which may be called 'layer of basal corpuscles' (fig. 2). These are minute, oval bodies appearing, in surface view, as small granules arranged in parallel rows, which are, in most cases, at right angles to the larger side of the distal cell-end (fig. 3). Below the layer of basal corpuscles is another zone of dense nature, which stains more deeply with plasma-dyes. Through this zone, which provisionally may be designated 'transparent zone,' fine fibers pass vertically from the basal corpuscles towards the cytoplasm proper, where they become lost to view. The cytoplasm contains a large number of tortuous mitochondrial filaments, stained black by iron-haematoxylin; they are mainly arranged vertically and are accumulated especially in the upper portion of the cell-body, under the transparent zone, without passing

into it. No centrosome and mitotic figure were seen in the cells in question.

2. *Gills of Anodonta*. The epithelium consists of ciliated and non-ciliated cells. Of the ciliated cells I have studied lateral-cells and corner-cells. Both these cell-varieties are provided with a cuticle carrying long cilia, which, in fixed preparations, are curved towards the external surface of the gill-torus, as noticed by Engelmann ('80); this is especially well marked in the lateral-cells. The behavior of the layer of basal corpuscles, of the transparent zone and of the chondriocentes is analogous to that described above for the foot of the same species (fig. 4).

The layer of basal corpuscles, which seems to correspond to the zone found by Wallengren ('05) below the cuticle, contains basal corpuscles, in the shape and arrangement of which the lateral- and corner-cells differ widely from each other. Viewed from the surface (fig. 5 a), the lateral cells, each of which has a regular rectangular outline, are arranged in stone-wall-like rows running along the gill-torus. The basal corpuscles are spherical granules, which form parallel rows arranged in the direction of the short diameter of the cell-border, as is the case with the foot-epithelium of the same species. Engelmann ('80) has also described such a linear arrangement of basal corpuscles; he states that the lines are inclined about  $45^{\circ}$  to the longer margin of the cell-border, but I could not confirm this.

The corner-cells, separated from the series of lateral-cells by two rows of non-ciliated cells, are closely apposed on their flat faces, forming a series along the gill-torus (fig. 5 b); in surface view of this type of cell two parallel lines, stained black by iron-haematoxylin, run along the longer axis of the cell-border. These lines, named by Engelmann *bandelets* ('*Leistchen*') serve for the attachment of the cilia, and might conceivably be formed, as the author suggests, by the fusion of basal corpuscles.

3. *The intestine of Anodonta* (figs. 6-8, 16). The epithelium in this region consists of ciliated, non-ciliated and glandular cells. The first two of these are provided with striated cuticular borders; the clear space between the striae in lateral view correspond with pores seen in surface views; this suggests strongly that the

cuticular substance is perforated by canaliculae, filled with fluid; the striae are therefore nothing but the septa of these canaliculae viewed in profile. The cilia pass through the striae and come into connection with the basal corpuscles underneath the cuticle. The basal corpuscles, spherical or oval in form, are here also arranged in linear series running, in most cases, parallel to the shorter diameter of the cell-border. Since it seems that the larger diameter of most epithelial cells is oriented in a direction, perhaps parallel to the axis of the intestine, it is natural that the rows of basal corpuscles all run in a direction, which is at right angle to the axis of the intestine, a condition which, I think, is of an important significance for the function of ciliated cells in this situation. Under the layer of basal corpuscles follows a comparatively narrow, transparent zone, through which fine fibers, arising from the basal corpuscles, pass downwards.

The chondriocotes in the cytoplasm are chiefly arranged in a vertical direction and are largely accumulated beneath the transparent zone (figs. 6, 8). They seem not to be floating in the plasma-sap, but to be suspended on or imbedded in the beams of the plasma-network. If a reagent which causes a shrinking of the cell, such as sublimate-mixture, be employed (fig. 7) there appears a sinuous or spiral fiber, stained deeply by iron-haematoxylin, and splitting upwards into several filaments, which end under the transparent zone. With Meves's fluid, on the contrary, no such feature appears (figs. 6, 8). From this it is highly probable that this fiber, found by various investigators and termed 'cone of rootlets,' is produced by the act of shrinking, which must result in the cohesion of beams of the plasma-network with the suspended chondriocotes. Centriosomes and mitosis are often met with in ciliated cells from this location.

4. *The middle-intestine of Helix.* The constituent elements of the intestinal epithelium of this species are the same as those of Anodonta. The ciliated cells (figs. 9, 15) bear comparatively short cilia, which pass through the cuticle and come into connection with basal corpuscles; these latter are very small granules, so closely applied to one another that they appear, at a certain



degree of differentiation of iron-haematoxylin, as a single dark line beneath the cuticle. The transparent zone is about one half the height of the cuticle and is marked off distinctly from the cytoplasm proper. Chondriocotes are also visible; their arrangement is similar to that described above for Anodonta. Centrosomes and mitotic figures are easily recognizable; two- or poly-nucleated cells also occur.

5. *The hepatic duct of Helix.* The epithelium in this region is composed of ciliated cells with interspersed glandular- and non-ciliated columnar cells; in approaching the gland proper non-ciliated cells increase in number. The cilia pass, as usual, through the cuticle in order to connect with basal corpuseles, which appear as minute granules and form, viewed from the surface, linear series running parallel with one another (figs. 10, 11). Sometimes the course of the chondriocotes is so regular, that they form a sort of palisade (fig. 10). In order to follow the fibrils which pass downwards from the basal corpuseles it is necessary to remove the chondriocotes, which often prevent close examination. This is realized practically in poorly preserved portions of the sublimate or Meves materials. Sections from such portions show that the fibrils in question (fig. 11) pass, in a slightly tortuous course, through the cytoplasm towards the distal end of the nucleus. These fibrils (corresponding with what are designated as rootlets of the cilia) appear not to be free, but to be connected with protoplasmic networks and are not to be confounded with the chondriocotes, which seem to be suspended on or embedded in the beams of the network and never pass into the transparent zone. Centrosomes and mitosis of ciliated cells occur not infrequently; two-nucleated cells also often are met with.

6. *The intestine of Helix.* There exist no noteworthy differences in structures of ciliated cells between the intestine of *Limax* and that of *Helix*, so that its description can be omitted.

7. *The epidermis of Amphibian larvae.* The epidermis of young Amphibian larvae is composed of two layers; in the upper or cuticular layer, there are two kinds of cells: non-ciliated and ciliated cells. The latter (figs. 27-32) are few in number and

are scattered over the epidermis. They are furnished, at the distal border, with striated cuticle; each stria corresponding with a cilium. With favorable staining the basal corpuscles appear as diplosome- or dumb-bell-shaped granules (figs. 31, 32); one of these granules is situated at the upper border, the other at the lower, of the cuticle; the former is always smaller and more easily decolorized than the latter, so that there often appears a single row of basal corpuscles below the cuticle. The axis of these diplosome-like basal bodies either corresponds with the vertical axis of the cell in question (fig. 32) or is inclined in a determinate direction (fig. 31). When studied in tangential sections, the basal corpuscles form parallel rows and are inclined towards one end of the row (fig. 33). Since the inclination of basal corpuscles occurs only in a determinate direction, it is evident that, when the cell in question is cut parallel to the rows, the feature as represented in figure 31 is produced, and, that, on the other hand, the same holds true for the cell with vertical basal corpuscles in the sections which are cut at right angles to that direction (fig. 32).

The chondriocotes in the ciliated cells are more numerous than in neighboring non-ciliated cells and are collected especially beneath the cuticular border. In case the cilia are localized in a certain circumscribed region, the main mass of the chondriocotes drifts to that portion, which indicates a close relation between the two (fig. 27). In sublimate preparations, fibers which, arising from the basal corpuscles, pass downwards, can be readily seen (fig. 30); they are not to be identified with the chondriocotes, but must be regarded, from their staining reaction and the continuity with basal corpuscles, as rootlets of the cilia.

8. *Tela chorioidea of Amphibia-larvae.* The ciliated epithelium-cells from this structure (figs. 36-38) carry comparatively thick cuticular borders, which are not homogeneous, but are finely striated in the longitudinal direction. The cilia, which may be located in a circumscribed portion, especially in the middle of the cell-border, pass vertically through the cuticle and are connected with basal corpuscles. The latter are situ-

ated in a layer corresponding to the transparent zone of the Meves preparates, and appear as diplosome- or dumb-bell-shaped granules; the distal granules are always small and readily decolorized. The axis of the basal corpuscles is not vertical, but is inclined to one side of the cell (fig. 38). Since, in other cases there are vertical basal corpuscles, it appears probable that here also there may be the same condition as in the ciliated epidermis cells of the same species. The corpuscles are, however, irregularly disseminated, without forming rows (fig. 39). In the cytoplasm there are numerous chondriocentes, which are often oriented towards the cilia, a condition which is especially striking in cases when the cilia are localized in a certain portion of the cell-border (fig. 36).

9. *The ciliated infundibulum of Amphibia-larvae.* The ciliated cells (fig. 41) are short, cylindrical in shape and covered with thin structureless cuticular borders. The cilia are very long and often adherent into a thick curved bundle, the free end of which is always directed towards the peritoneal canal. The cilia penetrate the cuticle and come into connection with the basal corpuscles, which appear, in tangential sections of sublimate-materials, as very small granules scattered irregularly over the cell-surface, with the exception of a narrow peripheral zone. The chondriocentes are mainly accumulated in the distal portion of the cell-body, separated, however, from the layer of basal corpuscles by the transparent zone.

10. *The pharynx and oesophagus of Amphibia (figs. 42-55).* The cuticular border of the ciliated cells is longitudinally striated, each stria corresponding to a ciliary fiber. Since the basal corpuscles are very small and set closely side by side, it is difficult, in side view, to distinguish between them (fig. 46). In tangential sections they form linear series, parallel to one another, the corpuscles in the same row being united by a slightly staining fiber (figs. 47, 50); the lines lie, in most cases, in a direction at right angles to the longer side of the cell-border. In the transparent zone there are fibrils which run from the basal corpuscles downwards and are to be identified with the rootlets of the cilia. The chondriocentes are mainly arranged vertically and

are accumulated especially underneath the transparent zone. Ciliated cells with a centrosome and those with two or more nuclei often occur.

11. *The gall-duct of Amphibia.* The epithelium of the gall-duct of *Amphibia* consists of ciliated and non-ciliated columnar and basal cells. The ciliated cells (fig. 57) are columnar in shape and bordered with a cuticle, through which the cilia pass to connect with the basal corpuscles. These are small granules, situated underneath the cuticle. If, as in a certain degree of differentiation is the case, the points where the cilia are connected with the upper border of the cuticle appear as black granules, then the feature of diplosome- or dumb-bell-shaped corpuscles would be brought about. The behavior of the transparent zone and of the rootlets of the cilia is about the same as described in the pharynx of *Amphibia*. Ciliated cells with two nuclei and those with centrosomes are not infrequent here.

12. *The oviduct of Amphibia.* The oviducal epithelium is composed of ciliated and glandular cells. The former (figs. 63, 66) have thin cuticular borders; the basal corpuscles appear either as short rods in the cuticle, or as small granules underneath it. This difference of shape may perhaps be correlated partly with the degree of staining, partly with different animals. Thus I have found short, rod-like basal corpuscles in the oviduct of *Triton*, whereas they appear in that of the frog as of minute granules underneath the cuticle. In tangential sections they are arranged in parallel rows, all of the granules of one row being united by a fine slightly-staining thread. The direction of the rows corresponds to that of the short side of the distal cell-border (fig. 67). The chondriocentes are mainly gathered under the cuticle, the transparent zone is illy defined; binucleate ciliated cells often occur.

13. *Ciliated cells in the peritoneum of Rana temporaria.* The occurrence of ciliated cells in the peritoneum of female frogs was first described by Thiry ('62), and then confirmed by Schweiger-Seidel and Dogiel, Neumann, Nussbaum and others. According to these authors the ciliated cells in question are flattened and are smaller than neighboring endothelial cells; they are either



scattered or form rows. The type of cell which I have found among the peritoneal cells near the oviduct is represented in figure 68. The cilia are comparatively thick and are connected with spherical basal bodies; a special cuticular border seems to be wanting in this cell.

14. *The trachea of Trigonocephalus.* The ciliated cells in this part (fig. 69) have on their free surface, cuticular borders and relatively long cilia. The basal corpuscles are differentiated with difficulty, and therefore appear in most cases as a dark line underneath the cuticle. In tangential sections they appear as minute granules, closely applied to one another, the whole, at first sight, resembling a fine network. On close inspection, however, I had no difficulty in making out linear series of granules, arranged in a determinate direction. The transparent zone and the chondriocontes behave in the same manner as described in other species. Ciliated cells with two nuclei are not infrequent.

15. *Efferent tubules of the testis of Reptilia.* Of the reptiles, have studied snakes, adders and lizards. The epithelium consists of ciliated cells and cells with brush borders. The former are small in number in proportion to the latter. At the free extremity of the ciliated cells (figs. 72, 73, 76) there is a narrow zone, darkly stained by iron-haematoxylin and presenting the appearance of a cuticular border. The basal corpuscles are either imbedded in this zone or situated beneath it. The chondriocontes are collected below the cuticle, being separated from it by a narrow transparent zone. Binucleate ciliated cells often occur in the efferent duct of these forms.

16. *The oviduct of the lizard.* The oviducal epithelium consists of ciliated and glandular cells. The former are few in number as compared with the latter and bear on their free surface thin cuticular borders. The basal corpuscles are in most cases rod-like granules imbedded in the cuticle; in other cases they appear as small spherical bodies beneath the cuticle. This difference may perhaps be connected with the degree of staining. The transparent zone is illy defined; binucleate ciliated cells are not rare.



17. *Efferent ducts of the rat and mouse.* The epithelium consists of ciliated cells and cells with brush borders. The ciliated cells (figs. 84-88) taper gradually to their proximal extremity. Their nuclei are large and take a higher position than those of neighboring cells, as already noticed by Lenhossék (1898). The uppermost portion of the cell-body generally is of a dense character, and basal corpuscles, rod-like, diplosome- or dumb-bell-shaped, are arranged here in parallel rows. Any well-defined cuticular border, such as seen in other places, is not discernible here. There are centrosomes in the transparent zone. The chondriocotes are mainly collected below the transparent zone, without entering into it. The basal corpuscles, viewed in tangential sections, are not scattered irregularly, but are arranged in linear series, parallel to one another and at right angles to the larger diameter of the cell-border. There is always a single nucleus.

18. *The trachea of the rat and mouse.* The ciliated cells of the trachea of these forms (figs. 91-95) are bordered with a distinct cuticle through which the cilia pass downwards to connect with basal corpuscles. These are small spherical granules situated under the cuticle; in a surface view their linear arrangement is conspicuous (fig. 95). The chondriocotes never enter into the transparent zone. The cells are often provided with two nuclei, the centrosome is readily discernible.

#### B. GENERAL CONSIDERATIONS

##### a. *The manner of implantation of the cilia*

Ciliated cells are either columnar, or cubical, or, in rare cases, flattened in shape, and, although they participate in the formation of the simple or stratified epithelium, it can hardly be said that there is any epithelium which consists of ciliated cells only, other kinds of cells, such as non-ciliated columnar or glandular cells almost invariably entering into its composition. And while the ciliated cells, on the one hand, are partly eliminated after degeneration, partly converted into columnar or glandular cells, the columnar epithelial cells, on the other hand, become trans-

formed into ciliated cells. This is a physiological change which is going on, not only in embryonic or young tissues, but also in those which are fully developed. Under these conditions, the differentiation of the superficial plasma-portion of a ciliated cell might be considered from two morphological points of view: first, from the structures derived from the normal columnar cell, and then from what are newly formed with the development of the ciliary apparatus; and it is natural that these differentiations vary according to the forms of epithelial tissues in which the ciliated cells are involved.

It is extremely rare that the ciliated cell exhibits no special differentiation in its distal border. Even in such cases as where a distinct cuticle seems to be lacking, as Lenhossék ('98) and Studnička ('99, '00) state, the distal border of the ciliated cell where the basal corpuscles lie, is somewhat dense in character and stains more heavily with plasma-dyes. This fact might have, as Lenhossék remarks, a certain amount of importance for the physiological function of the cilia.

1. *Relation between the cilia and the cuticle.* Friedreich ('58) was the first to describe the cuticular border of the ciliated cell; he found a striated cuticle in the ciliated cells of the trachea of man and bull, and of the ventricular wall of the human brain; and believed that the cilia pass into the cytoplasm along the striae. The structure in question has since been noticed by Eberth ('66), Marchi ('66), Engelmann ('68) and others.

I find in the literature of the subject five different opinions with regard to the structure of the cuticular border and the manner of implantation of the cilia:

1) Rabl-Rückhard ('68) asserts that, although he found a cuticle in the ciliated cells of gills of Annelida, the cilia never pass through it, but are attached to its upper surface by somewhat dilated bases.

2) Friedreich ('58), Stuart (67), Apáthy ('97) and Gurwitsch ('01, '04) believe that the ciliated cell has a homogeneous cuticle, through which the cilia pass into the cytoplasm, and, in addition, it was shown by Gurwitsch that the cuticle must be soft, since the cilia pass through it irregularly.

3) According to Engelmann ('80), Gaule ('81), Carrière (82), Heidenhain ('99) and others, the cuticle is provided with rod-like corpuscles, which appear, in profile, as longitudinal parallel striae, with the upper ends of which the cilia are connected; a view which is not markedly different from that described under 2).

4) Marchi (66) and Studnička ('00) admit sieve-like perforations of the cuticle, through which the cilia pass downwards into the cytoplasm.

5) This view admits an alveolar structure of the cuticular border. Gurwitsch ('01) finds that the cuticle, in surface views of the ciliated cells from the rabbit's oviduct, is made up of regularly arranged alveoli; at the nodes of the alveolar networks there are basal corpuscles, with which the cilia are connected. Studnička ('00) also found an alveolar structure of the cuticle in the ependyma cells of *Spinax niger*.

I have, in turn, found, in all forms studied, that the superficial portion of protoplasm of the ciliated cell, that is to say, the part where the cilia are implanted in the cell, is always of a denser character, appearing either as a crust or as a well-defined cuticle, which varies greatly in thickness.

Whether the longitudinal striation of the cuticular border, seen in profile, is a feature brought about by the passage of the cilia, or is owing to rod-like structures in the cuticle, or is an appearance due to canaliculi, or is produced by an alveolar structure of the cuticular border, is a question which is very difficult to solve; at least when one attempts to draw any conclusion from the study of ciliated cells themselves, since the feature is more complicated by the implantation of the cilia. I believe that the clue to the solution of the problem is furnished by the study, either of those cases in which the cilia are limited in a certain circumscribed portion of the cuticle (e.g., in the epidermis and tela of *Amphibia-larvae*), or in the transformation of non-ciliated into ciliated cells, or in the redifferentiation of ciliated cells into non-ciliated. From such studies I have been able to find three types of differentiation of the superficial portion in the ciliated cells studied:

1) In the first type, such as in efferent tubules of the testis of Reptilia and mammals, I have never been able to recognize any well-defined cuticle; what can be seen is an ill-defined crust, in which the basal corpuscles lie, thus giving rise to the appearance of a longitudinal striated cuticular border. That this appearance is owing to the existence of basal bodies in the crust, is readily explained by the study of ciliogenesis in epididymis cells of mammals. In efferent tubules of the mouse, as will be afterwards described, it is evident that cells with brush borders, which are provided with a crust on their distal margin (fig. 89), become converted into ciliated cells, and that from the way that the newly-formed basal corpuscles arrange themselves in the crust. In one word, the striated cuticle in this case is nothing but a portion of the crust defined by the arrangement of basal bodies.

2) To the second type belong the ciliated cells of the epithelium of the pharynx and tela of Amphibian larvae. As the representative of this group I take the ciliated cells of the epidermis, the most closely examined. As will be afterwards described, these ciliated cells are developed from non-ciliated, the cuticle of the latter becoming directly converted into that of the former. The cuticle, in profile longitudinally striated, exhibits, in surface view, a well-marked reticular appearance. If these two features are considered associated with each other, it becomes evident that the cuticle is composed of closely apposed alveoli, the junction of the alveolar walls appearing, in side view, as striae (compare: F. E. Schulze '69, '88; O. Schutze '07; S. Saguchi '15), through which the cilia pass into the cytoplasm.

The structure of the cuticle of ciliated ependyma cells is the same as that above described, except that the longitudinal striae are very closely applied, rendering close examination extremely difficult (figs. 36, 38). Studnička ('99) describes the cuticle of ciliated cells in the tela epithelium of *Petromyzon* as provided with small canals through which the cilia pass into the interior of the cell. This seems, however to me to be an artificial result brought about by the fixation; for but one of the seven cells in his figure 5 has such canaliculi, and he says in his text that "sie (canaliculi) sind, wie es scheint, viel grösser als es zu diesem Zweck (passing through of the cilia) nötig wäre. . . ."

His figure 5 to 7 and 8, rather, appear to me evidently to show an alveolar structure of the cuticular border.

3) The intestines of *Helix*, *Anodonta*, *Lumbricus* and *Limax*, and the hepatic duct of *Helix* belong to this group. Before proceeding to describe the ciliated cells it will be necessary to say a few words with regard to the structure of the cuticle of non-ciliated intestinal cells. There are three opinions with regard to this question: (1) the cuticle is perforated by small vertical canals (Köl liker, Funke); (2) it is provided with rods (R. Heidenhain); (3) it is composed either of coarser or of slender, finger-shaped rods (Studnička). In intestinal cells of the invertebrates studied, I have found that the cuticle has, viewed in profile, an appearance of longitudinal striation, seemingly caused by a parallel arrangement of rods; the clear vertical narrow lines correspond to the spaces between them. In surface view, minute, round, clear pores appear scattered over the cuticle. These facts point to the canalization of the latter, as Köl liker and Funke maintain.

The structure of the cuticle of ciliated cells is the same as that of the non-ciliated and can readily be observed in the functional change of the former, that is to say, in their transformation into non-ciliated or glandular cells (figs. 8, 11). In these figures it can well be seen that the cilia never pass through the canaliculi, but through the axis of the rods themselves.

The intestinal cell has been considered by some to have a brush border, and there is much diversity of opinion in regard to the relation between the cilia and the hairs of the supposed brush border. Vignon ('00) found, in the intestine of *Chironomus* larvae, that the cilia are attached to the free extremities of the hairs of the brush border, and believes that there is no genetic connection between these two structures, whereas Pre-nant ('97-'99) and Holmgren ('03) assert that the hairs of the brush border are nothing but atrophied cilia. According to Gurwitsch ('01), there exists no relation, either genetic or anatomic, between the cilia and the hairs, the former passing independently through the spaces between the latter.

2. *Relation between the cilia and the brush border.* Of the ciliated epithelia studied, I have found cells with brush borders in



only the efferent tubules of the reptilian and mammalian testis. The cells with brush borders in these places, as will be afterwards more particularly described, become converted into ciliated. In this transformation the cilia are produced, not by the lengthening of the hairs, but from an intracellular constituent, which passes out through the axis of the hairs, so that there exists no genetic relation between the two. I will return to this question further on.

3. *The basal corpuscles.* Historical. By the basal corpuscle is meant a minute granular or rod-like body, situated at the proximal end of each cilium and stained black by iron-haematoxylin. It would seem never to be lacking in the ciliated cell proper; there is, however, no uniformity as to its shape and position; it also varies considerably according to the degree of coloration.

A review of the literature shows that there is a considerable difference of opinion as regards the shape, position and arrangement of basal corpuscles, which is briefly summarized as follows:

Shape: (a) spherical or elliptical granules (Lenhossék '98, Studnička '99, Wallengren '05, Erhard '10); (b) short rod-like corpuscles (Studnička '99, Ikeda '06); (c) dumb-bell-shaped or diplosome-like granules (Gurwitsch '00, '01; Henry '00; Fuchs '04; Koláčev '10; Tschassownikow '13).

Position: the basal corpuscles are situated (a) in the distal border of the ciliated cell destitute of the cuticle (Lenhossék '98); (b) in the cuticle itself (Heidenhain '99, Gurwitsch '01); (c) underneath the cuticle (Apáthy '97, Studnička '99, Wallengren '05, Erhard '10); (d) more or less deeply in the cytoplasm below the cuticle, as in the ciliated cells in the tela of *Petromyzon* and *Salamandra* (Studnička '99).

Arrangement of basal corpuscles; viewed from the surface: they are either (a) irregularly scattered (Lenhossék '98); or (b) gathered in the central part (Studnička '00); or (c) arranged in linear series (Engelmann '80, Gurwitsch '01); or (d) fused into lines (Engelmann '80).

*Summary and discussion.* It is worthy of remark, that the result of staining with iron-haematoxylin, which is usually applied

in order to exhibit the basal corpuscle, varies according to the grade of differentiation. This is an advantage, and, at the same time, a difficulty, of that technic. To speak more correctly: while, on the one hand, it enables one to judge the intensity of its affinity for cell-constituents, the same constituent of the cell, on the other hand, often presents a good deal of variety according to the degree of staining, which may give rise to misinterpretation. Not only the manipulation of staining, but also the degree of maturation of haematoxylin-solution used has a marked effect upon the result. An especially important factor in this staining is the method of fixing and the quality of fixative. For example, in preparations from materials perfectly fixed in Flemming's fluid the mitochondrial filaments stain deeply, whereas the basal corpuscles remain unstained; in poorly-fixed Flemming and in the sublimate-material, on the other hand, it shows an inverse staining capacity.

So far as can be seen from my preparations, the basal corpuscles may be classified according to their shape and position as follows:

1) Spherical or elliptical basal corpuscles which are situated beneath the cuticle; these forms are met with in the gill (fig. 5, lateral cells), foot (figs. 1-3) and intestine (figs. 6-8) of *Anodonta*, in the intestine (fig. 9) and hepatic duct (figs. 10, 11) of *Helix*, in the intestine of *Limax* and *Lumbricus*, in the pharynx of *Rana*, *Bufo* and *Triton* (figs. 46, 49), in the gall-duct of *Rana* (fig. 57), and in the trachea of *Trigonocephalus*, of the mouse and rat (fig. 92). Such cases as this are found in the description and figures of the following authors who studied the intestines of invertebrates: Apàthy ('97, typhlosole of *Anodonta*, Lenhossék ('98, middle intestine of *Anodonta*), Heidenhain ('99, intestine of *Helix*), Gurwitsch ('01, intestine and mouth of *Lumbricus*), Holmgren ('03, pharyngeal pouch of *Lumbricus*), Erhard '10, typhlosole and hepatic duct of *Anodonta*), and Koláčev ('10, intestine of *Anodonta*).

The corpuscles found by Engelmann ('80) in the intestine of *Cyclas* and *Unio*, and named by him 'Fussstücke' may, as already stated by Studnička ('99), be identified with the basal corpuscles. Heidenhain ('99) describes and figures lozenge-

shaped basal bodies at the distal margin of the cell; Brasil ('04) has found in ciliated cells of the middle intestine of *Lagis coreni*, rod-like corpuscles, termed 'La bâtonnet cilifère,' which, to my mind, are not special rods, but correspond with the intracuticular portions of the cilia.

As to the ciliated cells of the gills of Anodonta, my observations are in accord with those of Wallengren ('05) and Erhard ('10).

The basal corpuscles in the ciliated cells of the Amphibian pharynx are, so far as can be seen from my materials, extremely small, often appearing in profile as a dark line beneath the cuticle, a feature which has been already noticed by Lenhossék ('98). In all of my preparations which are stained to varying extents, I have not been able to make out rod-like or dumb-bell-shaped basal corpuscles in the cuticle, as described by Eimer ('77) and Studnička ('99) in the pharynx of *Salamandra*, by Gurwitsch ('01) in the oesophagus of *Bufo*, and by Tschassownikow ('13) in the oesophagus of the axolotl. I am not quite sure whether this difference is due to the materials used or to other factors.

2) Dumb-bell-shaped or diplosome-like basal corpuscles. These are situated (a) in the crust, as in the ciliated cells of efferent tubules of the mouse and rat (figs. 84-88); (b) in the cuticle, as those of the Amphibian epidermis (figs. 31-33); (c) beneath the striated cuticle, as in those of tela of *Amphibia* (figs. 37-38).

In the cases (a) and (b) the upper granules are easily decolorized, thus giving rise to the appearance similar to that described under 1).

Regarding the basal corpuscles in the ciliated cells of the efferent tubule, I refer to the papers of Lenhossék ('98), Henry ('00), Ach ('02), Fuchs ('02, '04), and Ikeda ('06). Of these authors, Lenhossék, Henry and Ikeda have found oval or short rod-like basal corpuscles, while Fuchs noticed, in addition to these, diplosome-like bodies.

My observations on the tela epithelium are entirely in accordance with those of Studnička ('99, '00) on the ciliated ependyma cells of *Cyclostoma*, fishes, *Rana*, *Salamandra*, and of man, and

with those of Gurwitsch ('01) on the ciliated cells of the tela epithelium of *Salamandra*.

3) Short rod-like basal corpuscles, which are imbedded in the thin cuticular or crust-like, distal border of the cell. The ciliated epithelial cells of the oviduct of *Rana temporaria*, *Triton*, and the lizard, and those of efferent tubules of the lizard, snake, and *Trigonocephalus* belong to this type (figs. 66, 73, 76).

As regards the arrangement of basal corpuscles in surface view, I can distinguish the following three types:

1) The basal corpuscles are gathered, without special disposition, in the middle of the cell; such a form is found in the ciliated cells of tela chorioidea of Amphibian larvae (fig. 39) and corresponds with what Studnička ('00) noticed in the ciliated ependyma cells of *Cyclostomata*, *Rana*, and especially of man.

2) They are arranged in linear series, parallel to each other, and in most cases at right angles to the longer side of the cell-border. I have found such a type in the following situations: in the gill (fig. 5 a), intestine (fig. 16,) and foot (fig. 3) of *Anodonta*, in the hepatic duct of *Helix*, in the epidermis (fig. 33), pharynx (figs. 47, 50), and oviduct (fig. 67) of *Amphibia*, in the trachea (fig. 95) and the efferent tubule (figs. 83, 90) of the mouse and rat.

That the view of Engelmann ('80) as to the arrangement of the basal corpuscles in ciliated cells of gills of *Anodonta* is at variance with that of my own, has been already mentioned in the foregoing chapter.

For the surface view of ciliated intestinal cells of invertebrates I refer to the figures of Heidenhain ('99) from the hepatic duct of *Helix*, of Gurwitsch ('01) from typhlosoles of *Lumbricus* and *Anodonta*, and of Koláčev ('10) from the intestine of *Anodonta*. According to the figure of Heidenhain, the rows of basal corpuscles are nearly at right angles to the longer side of the cell, while Gurwitsch figures rows parallel to the latter. This latter type I have seen very rarely, but never such a type as Koláčev figures in which the basal corpuscles are irregularly scattered over the cell-surface.



The figures of Henry ('00) of the ciliated cell of the epidermis, coincide in many points with mine, whereas in Lenhossék's ('98), Fuch's ('04) and Ikeda's ('06) figures we find no definite arrangement of the basal corpuscles.

3) It will be seen from the foregoing that the basal corpuscles show a marked tendency to form parallel rows and to undergo fusion with each other; and in fact, my attention was repeatedly attracted, in some places (in the oviduct of Triton, in the pharynx of Amphibia, in the foot of Anodonta) by the presence of a faintly staining fibril, uniting the basal corpuscles of the same row, as Heidenhain ('99) shows in his figure from the hepatic duct of Helix. The case which may be regarded as the extreme of this process is found in the corner-cells of gills of Anodonta (fig. 5 b). In flat view of this cell as mentioned above, there can be easily found two parallel stripes, which are stained black by iron-haematoxylin, and from which the cilia arise. Regarding the significance of these stripes Engelmann ('80) expresses himself as follows: "Die Cilien entspringen oben auf jeder Zelle von zwei, den langen Rändern parallelen Leistchen, die nichts anders als die verschmolzenen, oder richtiger reihenweise aneinander gefügten Fussstücke der elementalen Cilien," a view with which I agree entirely. The stripes, for which it will be better to reserve the name 'basal stripes,' are not to be identified with 'Basalkörperfasern,' found by Kuperweiser ('06) in the ciliated cells of the corona of Cyphonautes-larvae; for the cilia never arise from these fibers.

4. *The so-called basal rods (Frenzel's Fussstübchen).* As early as 1877 Eimer noticed, on the upper border of the ciliated cells from the pharynx of Salamandra and from the gills of Siredon pisciformis, the existence of rod-like corpuscles, the juxtaposition of which gives rise to the appearance of a cuticle. Three years later Engelmann described strongly refracting rods on or in the distal border of the ciliated cells from intestine of Cyclas and from the nasal mucous membrane of the frog, and termed them 'Fussstücke.' These rods are, according to his description, attached by means of the intermediate segments (Zwischenglieder) to the hair-bulbs. From these observations, the



author claims, with Eimer, that the cuticular border of the ciliated cell, as seen by Eberth, Marchi and many others, is nothing but the juxtaposition of the 'Fussstücke.' Similar bodies were afterwards found by Gaule ('81) and Frenzel ('86). Especially the latter author recognized the presence of Engelmann's 'Fussstücke' in ciliated cells from various classes of invertebrates and gave the name of 'Fussstäbchen' to them. According to him, the length and the structure of these corpuscles vary in various animals and situations. In the simplest case it consists of a rod with a small knob at one or both ends, but the most complex one he found in the intestine of *Littorina* and *Rissoa*, which is composed of the following five segments: (1) the lower knob, (2) the basal block, (3) the side-knob, (4) the rod, and (5) the upper knob.

It is difficult at the present time to harmonize the 'rod theory,' urged by Engelmann, Frenzel and others, with the so-called 'cuticular theory' of Eberth and Marchi; for the same structure is materially influenced by the technique and by the character of the optical instruments used. Most of the recent investigators (Schiefferdecker '91, Apáthy '97, Lenhossék '98, Heidenhain '99, Studnička '99, '00, Gurwitsch '01, Wallengren '05, Kuperwieser '06, Erhard '10, Tschassownikow '13) have found a well-defined cuticle in the ciliated cell, through which the cilia pass. Lenhossék especially expresses himself as follows:

Der Versuch von Engelmann und Frenzel, die Existenz einer eigentlichen Cuticula in Frage zu stellen und das, was man als solche beschrieben hat, bloss als das Ergebniss der mosaikartigen Zusammenlagerung der vertikalen basalen Teile der Flimmerhaare zu erklären, muss als verfehlt bezeichnet werden. Die 'ältere Cuticulartheorie,' wie sie Frenzel nennt, ist noch immer richtige.

Brasil ('04) still maintains, in the ciliated intestinal cells of *Pectinaria*, the existence of rod-like basal differentiations of the cilia, which correspond, according to him, to the basal rods of Frenzel. Studnička ('99), on the other hand, finds the cuticle in some cases, in others not, but an arrangement of rod-like corpuscles.

I have, in my turn, noticed in all ciliated cells studied, as the product of differentiation at the distal cell-border, a crust or striated cuticle provided with basal corpuscles or penetrated by the cilia, but I have never seen rod-like corpuscles situated on the outside of the cell-border. The cilia, from the basal corpuscles to their distal extremities, whether they are within or outside of the cuticle, show no trace of structure, but appear completely homogeneous. In the following I discuss the value of the 'rod theory,' comparing my results with those of Engelmann and Frenzel.

Engelmann ('80), (1) in his figures 11 a and b, 12, 15, shows a well-defined, homogeneous border on the row of his basal knobs; (2) in his figures 5, 9, 18, and 19, a row of basal knobs, the upper and the lower ends of which are defined by straight lines, so that the appearance of a striated cuticle occurs; (3) in figure 20, basal knobs arranged on the surface of a cell from the olfactory pit of *Rana temporaria*; the upper ends of these bodies are connected, by means of intermediate segments, with the hair-bulbs of the cilia. Thus Engelmann admits three types of implantation of the cilia. Is it rational to believe such a variety of structure in cells which fulfill the same function? I will try to account for this variety of structure in the following manner: In the first case Engelmann's conclusions are nearly in agreement with mine; for, in his figures, it is clearly seen that the cilia pass through the cuticle superposed upon the row of basal knobs which seem to correspond to my basal corpuscles. In the second case the author, in all probability, saw a striated cuticle with or without basal corpuscles. In the third case the cuticular substance, for some reason or other, escaped his observation, while the striae or the rod-like basal corpuscles, which stand out sharply, he takes to be independent rods upon the cell-border. The basal rods of Frenzel, on the other hand, seem to be distinct from the basal knobs of Engelmann in shape and refrangibility, as Frenzel says. I am rather, with Studnička ('99), of the opinion that the basal knobs of Engelmann correspond to the lower knobs of Frenzel. The hair-bulb of the above authors might be identified with the node produced by

the accumulation of plasma at the point of union of the cilium with the upper border of the cuticle, a view which has been held by Lenhossék ('98), Wallengren ('05) and Erhard ('10). These nodes are not only characteristic of ciliated cells, but also seen in ordinary epithelial cells; their existence in the epidermis cells of *Amphibia-larvae* has been shown by Leidig ('85) and Studnička ('98).

From the above the inference would appear justifiable that the adherents of the 'rod theory' failed to notice any structural difference between the substance of the cuticle and that of the ciliary apparatus, but considered the more readily recognizable parts as a continuous structure, and overlooked the rest, a result either of poor fixation or of studying fresh tissues.

#### *b. The cytoplasm and rootlets of the cilia*

In the literature there are three types of special structure of the interior of the ciliated cell:

1) Valentin and Buhlmann first noticed the longitudinal striation of the cytoplasm, which has since been recognized by Friedreich ('58), Eberth ('66), Marchi ('66), Eimer ('77), and especially by Engelmann ('80), and named by the last author 'rootlets of the cilia.' And besides these, Lenhossék ('98), Fischer ('99), Peter ('99), Heidenhain ('99), Gurwitsch ('01), Joseph ('03), Kuperwieser ('06) and many others have found that the rootlets of the cilia are connected, above, with the basal corpuscles; below, they either lose themselves in the neighborhood of the nucleus or descend, forming a conical bundle, to the base of the cell. Apáthy ('97) and Metalnikoff ('00), on the contrary, look upon this conical bundle as the termination of nerves which are not connected with the basal corpuscles, but which end freely between them.

2) According to Lenhossék ('98) and Fuchs ('02), the ciliated cells of the epididymis are devoid of rootlets of the cilia, but they contain, in the upper portion, granular substance which Fuchs takes to be mitochondria.

3) Neither rootlets nor granules are visible (Lenhossék, '98) in the trachea of the rabbit; (Gurwitsch, '01), in the oviduct of the rabbit; (Tschassownikow, '13), in the oesophagus of the axolotl.

I have been able to distinguish the following two plasma divisions in all the ciliated cells studied:

a) Under the cuticle there is a dense, transparent zone (figs. 6-9, 42-45), which is marked off below from the cytoplasm proper by a relatively distinct boundary-line. Apparently it never is lacking in ciliated cells, though it varies greatly in thickness. The rootlets of the cilia, arising from the basal corpuscles, pass through this zone (figs. 6, 54). Its existence was noticed by Gurwitsch ('01) in the ciliated cells of the pharynx of *Bufo*, of the intestine of *Lumbricus* and of the tela chorioidea of *Salamandra*, and we also find the same in the figures of Heidenhain ('99), and Erhard ('10). As stated above, the basal bodies are usually situated, either within the cuticle, or between this and the transparent zone; they may occur occasionally within the latter, as in the ciliated cells of the tela of Amphibian larvae (figs. 37, 38).

b) The cytoplasm proper occupies by far the largest part of the cell-body; it exhibits a reticular or alveolar structure and contains a goodly number of mitochondria.

The size and arrangement of the meshes of the protoplasmic reticulum are not the same in different cells. We find a most striking instance of the longitudinal elongation of these protoplasmic meshes in the ciliated cells of the intestine of *Anodonta* and of the hepatic duct of *Helix* (figs. 6-11). In these cases the main beams run vertically and pass through the transparent zone to come into connection with the basal corpuscles. It is natural that these longitudinal striae, which correspond to what is described by various investigators as rootlets of the cilia, do not run independently, but are connected with each other by minute lateral fibrils. In this respect I agree entirely with Kolačev ('10), who says:

Auf diese Weise entsteht das typische Bild eines etwas in die Länge gezogenen Netzes mit Verdickungen an den Knotenpunkten, wobei die

Längsseiten der Schlingen dieses Netzes, die sich durch ihre Massivität und ihre intensive Färbung auszeichnen, die Wurzeln der Flimmerhaare darstellen, während die Querbalken schwächer ausgebildet sind, infolge dessen sie nicht immer wahrnehmbar ist.

I have found the so-called cone of fibrils, which is regarded as a bundle of rootlets of the cilia in sublimate-preparations only, but never in Meves-preparations. From this it may be inferred that the cone is not a real condition, but an artificial product due to the shrinking effect of sublimate-solution, by virtue of which the rootlets of the cilia adhere into a bundle.

The mitochondria in the ciliated cells were first noticed by Benda ('99) in the mid-intestine of *Anodonta*, in the hepatic duct of *Helix*, etc. He thinks that the rootlets of the cilia are formed of mitochondria or chondriocotes. Fuchs ('02) says that, in the ciliated cells of the epididymis of the mouse, the region between the row of basal corpuseles and the nucleus stains darkly and contains, in Benda preparations, a large number of mitochondria. Perhaps a dark granular zone found by Lenhossék ('98) in the upper portion of the ciliated epididymis cell is a similar structure. Recently, Meves and Tsukaguchi ('14) have found that, in the ciliated cells of the small bronchi, the chondriocotes are accumulated for the most part in the superficial portion of the cell, some descending beside the nucleus.

This description of Meves and Tsukaguchi holds good for all the ciliated cells I have studied. The chondriocotes course chiefly in the direction of the cell-axis and are especially abundant beneath the transparent zone, without entering into it.

As regards the mutual dependence between the protoplasmic networks and the chondriocotes, I am fully justified in believing that the latter are not free, but are either imbedded in the trabeculae of the former or suspended on it; and, as the chondriocotes run mainly vertically and are suspended on the likewise vertical protoplasmic beams, that is to say, on the rootlets of the cilia, it often occurs that the latter are in a great measure hidden by the mitochondria; in fact, it would seem that these structures have not till now been distinguished from each other with certainty. Perhaps such granular or knotty appearance



of rootlets of the cilia, as are described by Eberth ('66), Gaule ('81), Lenhossék ('98), Benda ('99), Koláčev ('10) and others are produced by a linear arrangement of mitochondria; on the other hand, however, there is no reason for believing the existence of such straight rootlets free from nodes, as are figured by Apáthy ('97), Heidenhain ('99), and Erhard ('10).

#### IV. FUNCTION OF THE CILIARY APPARATUS

Although this complex and difficult problem has received much attention, it is still far from a satisfactory solution. The review of the literature concerning this is given by Pütter ('03), Erhard ('10) and Prenant ('14) to which the reader is referred for details. It may be briefly summarized as follows:

1) Some authors (Pütter, '03; Gurwitsch, '04; Erhard, '10; Koláčev, '10) admit an active mobility of the cilia, others (Peter '99) a passive.

2) As to the function of the basal corpuscles, some look upon them as a kinetic center of the ciliary movement (Henneguy, '98; Lenhossék, '98; Peter, '99; Joseph, '03), others as an end-organ of the nerve (Apáthy, '97), and still others as a supporting organ (Eismond, '00); Maier, '03; Kuperwieser, '06; Erhard, '10; Koláčev, '10).

3) The rootlets of the cilia are regarded (a) as exhibiting contractibility and mobility (Stuart, '67; Simroth, '76; Benda, '99); (b) as primitive fibrillae of the nerve (Eimer, '77; Apáthy, '97; Metalnikoff, '00); (c) as a nutritive organ (Engelmann, '80; Koláčev, '10); (d) as a supporting organ (Peter, '99; Eismond, '00; Maier, '03).

It is necessary to say here a few words as to whether protoplasm or nucleus takes part directly in the ciliary movement. Of course, all protoplasm may be endowed with contractility, but there is no reason for supposing that it causes directly the ciliary movement, except that it may furnish a fresh supply to the ciliary apparatus; otherwise such special cell-organs, as the basal corpuscles and the rootlets of the cilia, would lose their significance. This is sufficiently demonstrated in the experiments of Peter ('99) which show that even where the ciliated

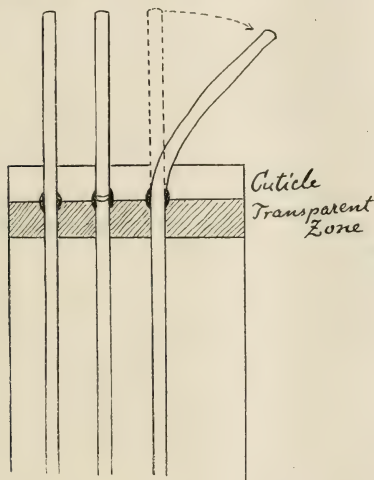
cell has lost its nucleus and the greater part of the cytoplasm, the cilia still continue to move, provided the ciliary apparatus be left intact.

The basal corpuscles, on the contrary, seem to play an important part in the ciliary movement and are never lacking in all true ciliated cells. Even when they have not been detected, it would be going too far to conclude that they are not essential to the movement of the cilia; for it often occurs that they are not brought out by staining. Moreover I cannot so disregard the basal corpuscles which Koláčev ('10) describes: "Für die Mechanik der Flimmerbewegung . . . ist das Vorhandensein eines fest fixierten Körperchens durchaus zweckentsprechend, wenn nicht gar physiologisch notwendig." The view of Apáthy ('97), postulating the nervous nature of the basal corpuscles and the rootlets of the cilia, is very curious and seems to have obtained few adherents amongst histologists.

Those investigators who accept an active mobility of the cilia themselves, claim to have observed, either the movement of broken cilia or of certain structures in them. Although I have studied sections from different places and with varying degrees of staining with iron-haematoxylin, I have never been able to detect either axis-fibrils, as seen by Koltzoff ('06) and Erhard ('10), or the transverse striation described by Koláčev ('10). In the following I, first, summarize my observations and then express my opinion concerning the ciliary movement: (a) There can be seen no trace of structure in the cilium; (b) at the basis of each cilium there is always a basal corpuscle, (c) beneath the row of basal corpuscles in most cases there can be seen a transparent zone, through which the rootlets of the cilia pass downwards from the basal corpuscles; (d) the rootlets of the cilia are only a part of the trabeculae of the protoplasmic reticulum; (e) the cilium, basal corpuscle and rootlet are a continuous structure; (f) viewed in tangential sections, the basal corpuscles are arranged in linear series, parallel to each other; the corpuscles in the same row show a marked tendency to undergo fusion with each other; (g) the dumb-bell-shaped or diplosome-like basal corpuscles form, in like manner, parallel rows. It often occurs that the upper extremity of every basal corpuscle

is inclined towards any one end of the row, and, at the same time, towards any one side of the cell; (*h*) in most cases these rows are at right angles to the longer side of the distal cell-border; (*i*) it seems that the direction of the rows agrees with that of the ciliary movement. These data lead us to the following conclusions:

- 1) The cilium itself has no active mobility.
- 2) The upper extremities of the rootlets are fixed in the compact, transparent zone, affording support to the cilia. I have



no reason for supposing that the rootlets may cause the ciliary movement.

3) I am inclined to believe that the kinetic center of the ciliary movement is to be sought in the basal corpuscles, as Henneguy and Lenhossék maintain, and in the following manner: it might be conceived that the basal end of the cilium and the distal end of the rootlet are connected either directly or by means of a joint, around which is the basal corpuscle (*s. text-figure*). The latter, like muscle-substance, is endowed with contrac-

tility, by virtue of which the cilium is bent at that point; hence the rootlet acts as a support of the cilium, while the transparent zone holds the rootlet in its position; the restoration of the bent cilium may be effected either by the elasticity of the cilium itself, or by the antagonistic action of the contractile substance. It must be remembered, in this connection, that Eismond ('00) compared the ciliary apparatus to the skeleton of the fin of a bony fish, but I am far from agreeing with him in regarding both the basal corpuscle and the rootlet as supporting organs of the cilium. And, as the ciliary movement usually proceeds in a determinate direction, it is natural that there should be linear arrangement of the basal corpuscles in that direction. With these conditions, a stimulation which causes the movement of the cilia, is transmitted in a waving manner from one end to the other of the same row, and thus the regular succession of the ciliary movement is attained; this must be looked upon as being most completely effected when all of the basal corpuscles of the same row are fused, as in the corner-cells of gills of *Anodonta*; the movements in different rows, on the contrary, are conceivably independent of each other.

## V. REGRESSIVE METAMORPHOSIS OF CILIATED CELLS

### A. TRANSFORMATION OF CILIATED CELLS INTO CELLS OF DIFFERENT NATURE

#### *Historical.*

That ciliated cells become converted into glandular cells has been observed by Knauff ('67), Flemming ('85), Osawa (97), and others in the trachea, and by Lenhossék ('98) and Tschas-sownikow ('13) in the pharynx of Amphibia. All of these authors state that droplets of secretion first appear above the nucleus, which becomes pressed downwards with the increasing accumulation of the former, so that at last there is formed a goblet or chalice cell. According to Lenhossék ('98) and Frenzel ('86), even in such glandular cells, the cilia can still be preserved and move actively. Next, the cilia are cast off and the secretion is discharged. The casting off of the cilia, according to Brasil

('04), results from the disappearance of the rootlets of the cilia on account of the accumulation of secreted material; concerning this he writes as follows: "La destruction du cône radicaire détruit les communications, quelle qu'en soit la nature, du cytoplasme et des cils. Isolés, ceux-ci ne peuvent plus que dégénérer et disparaître."

The transformation of ciliated cells into columnar has been observed by Henry ('00) in the epididymis, and by Gurwitsch ('00) in the intestine and the mouth of *Lumbricus*.

### *Observations*

1. *The hepatic duct of Helix.* The epithelium from this structure consists of ciliated, non-ciliated columnar, and glandular cells. The ciliated cells become converted into non-ciliated columnar and the latter into glandular. This process can easily be followed in one and the same section. First, minute granules, stained yellowish by iron-haematoxylin, make their appearance above the nucleus (fig. 11, the cell to the left); they then increase in size and number and pass gradually upwards. Next, the cilia and the basal corpuscles disappear (fig. 11, the cell to the right); it seems that the cilia are not withdrawn, but are cast off in one of two ways: (a) they are broken off at the junction with the cell; the broken fragments are, in fact, met with in large number in the lumen; (b) they curl up and become converted into small drops, which at first are applied to the surface of the cell, but afterwards are separated from it. Thus the ciliated cells are turned into non-ciliated columnar, and after the discharge of secreting mass into goblet cells.

2. *The intestines of Anodonta and Lumbricus.* The epithelium from these organs is composed of the same kinds of cells as those found in the hepatic duct of *Helix*. In many of the non-ciliated columnar cells there can be seen a row of basal corpuscles and a transparent zone, just as in the ciliated cells. On observing closely the cuticle of such cells, we often find some cilia (fig. 8) which are, as usual, connected with the basal corpuscles. Such cells might be interpreted as a form of transition between



the ciliated and non-ciliated cell, and as being on the road partly to the development, partly to the metamorphosis, of the ciliated cell.

3. *The pharynx of Amphibia.* Here some ciliated epithelial cells transform themselves into goblet cells; first, the cilia disappear and then the upper portion of the cell becomes narrower, in consequence of the pressure of neighboring cells, which may cause a bulging of the cuticular border (fig. 53); on the surface of the latter there can often be seen degenerated remains of the cilia. As the process proceeds, the lower half of the cell enlarges, and elongates downwards; the chondrioncotes gather into a conical heap, above the nucleus, secretion granules first being formed at the top of the cone. Such cases as this are, however, not frequently seen; most of the goblet cells, rather, seem to be formed by the proliferation of basal cells.

Lenhossék ('98) and Tschassownikow ('13) report that ciliated cells in the pharynx of Amphibia transform into goblet cells, and, that the cells may retain their cilia for a long time in spite of the accumulation of mucous droplets. Though I studied this point in considerable detail, I have not been able to confirm the latter opinion of these authors. I have frequently found some features which looked as if they might be due to the accumulation of mucous droplets in the upper portion of the ciliated cell, but, on closer inspection with higher power it became at once apparent that it was an appearance produced by the superposition of a tangential section of a goblet cell upon a neighboring ciliated cell, which may easily be understood when one recalls that the bulging goblet cell undermines neighboring ciliated cells.

4. *The oviduct of Amphibia.* The oviducal epithelium is composed of ciliated and glandular cells. The former become converted into the latter by the accumulation of secreted material; they retain their cilia for some time (fig. 65, *a* and *b*) before the discharge of the contents sets in, as stated by Lenhossék and Tschassownikow.

5. *The oviduct of the lizard.* The above statement for Amphibia holds good for this form (fig. 78).

*Summary*

1. Ciliated cells are transformed into non-ciliated columnar and glandular cells.

2. In the transformation of the ciliated into the glandular cell, the cilia disappear, either before or after the accumulation of secreted material.

3. The disappearance of the cilia is not by withdrawal but by their being cast off.

## B. ATROPHY OF CILIATED CELLS

That ciliated cells undergo atrophy, without being cast off, I have noticed in the gall-duct of *Rana temporaria*. The first change which occurs in the cell is nuclear hyperchromasy (compare my previous paper, 1915): there appear in the nucleus numerous nucleolar granules, stained black by iron-haematoxylin, just as the nucleolus. With the increase of these corpuscles in number, the nuclear sap comes to stain more and more darkly, so that at last the nucleus becomes entirely a black mass. The cytoplasm also sooner or later grows black, especially in its upper portion (fig. 58, 59). In the course of time the black mass grows more and more pale (fig. 60) and then there can be seen in the cytoplasm numerous, larger or smaller round or oval black corpuscles, which often are so numerous as to hide the nucleus entirely from view; the chondriocontes which were found before, have now disappeared. I think that the corpuscles in question are derived partly from degenerated chondriocontes, partly from the nucleolar granules passed out of the nucleus. While the cytoplasm still is in the state of heavy nucleolar hyperchromasy, the cell in question becomes constricted at the upper part, below the cuticle, two pieces, the upper smaller and the lower larger one, being thus produced (figs. 59, 60). Then the connection between the two becomes lost; the upper ciliated one is, in all probability, cast off into the lumen.

The ciliated corpuscles, first found by Neumann and then recognized by Schmidt ('82) must be remembered in this connection. These corpuscles, according to Neumann, bear cilia,

either in a circumscribed portion, or over the whole of the surface. Schmidt says that, though occurring numerously in the epithelium affected by catarrh, they are by no means infrequent in the normal epithelium; the author surmises their mode of development from the experiment, that, when ciliated cells are isolated, they undergo change in shape and constrict off spherical ciliated corpuscles. Since these bodies, according to his description, have no cuticular border, it must be regarded as a portion of the cytoplasm which has taken cilia with it. From this description and his figures, it would seem that the ciliated corpuscle of Neumann corresponds with the upper piece which is produced by the constriction of the atrophying ciliated cell. In contradistinction to the view of Schmidt, however, I cannot regard the corpuscle in question as derived from the cytoplasm only, but as composed mainly of the cuticular border. Similar corpuscles, but without cilia, were found by R. Heidenhain ('88) in the intestine of the rabbit, into which sulphate of magnesium was injected. From these facts it is evident that the constriction of the cell-body is a phenomenon which occurs in the degeneration, either of the non-ciliated columnar, or of the ciliated epithelial cell.

The lower piece which is left behind in the epithelium rounds itself off towards the basement membrane (figs. 61, 62), meanwhile the cytoplasm and nucleus become more and more clear and the black corpuscles are absorbed. The chondriocotes appear afresh in the cytoplasm (fig. 62), which indicates that the cell in question has not yet lost its vital activities. Thus the atrophied ciliated cell is transformed into a replacement cell; whether the latter returns to the ciliated cell, it is difficult to determine with certainty. Now the question arises, why are the cilia and the cuticular border cast off in the atrophy of the ciliated cell? I am inclined to believe that this phenomenon is owing to their intense functional specialization which incapacitates the structures from undergoing redifferentiation.

## C. ELIMINATION OF CILIATED CELLS

In the epithelium of the intestine of *Anodonta* it is often seen that some ciliated cells are being shed into the lumen. In the nuclei of such cells there appear numerous nucleolar corpuscles, while the nuclear sap becomes increasingly darker; that is to say, the nucleus is in the state of nucleolar hyperchromasy; in the cytoplasm, in addition, numerous larger or smaller vacuoles make their appearance, so that the whole exhibits a phenomenon of degeneration.

## VI. REGENERATION OF CILIATED CELLS

As the ciliated cells are reduced in number in the manner above mentioned, it is natural that they must be replaced by new ones. This is carried out either by the transformation of different kinds of cells into ciliated, or by the division of ciliated cells themselves.

## A. TRANSFORMATION OF OTHER KINDS OF CELLS INTO CILIATED CELLS

That ciliated cells are derived from replacement cells, was noticed by Drasch ('81), Flemming ('85), Bockendahl ('85), Joseph ('05), Tschassownikow ('13) and others. According to these authors, the mitotic figures which often occur in the ciliated epithelium, do not belong to ciliated cells themselves, but to cells of different nature, especially to replacement cells, which form a source of ciliated cells. Schnitzler ('93), on the contrary, admits the transformation of goblet cells into ciliated.

I have observed, in the efferent tubule of testis of the adult mouse and rat, that ciliated cells are derived from neighboring non-ciliated, with which process I shall deal later in the chapter on the development of the cilia.

## B. MITOSIS OF CILIATED CELLS

Although the question as to whether the ciliated cell multiplies by mitosis or not, has received much attention, it is still

far from a satisfactory solution. From the well-known fact that the centrosome directs mitosis in general, it must be admitted that the mitosis of a ciliated cell is also in close relation to the existence of the centrosome.

*The centrosome of the ciliated cell*

Upon this question, two distinct and opposed views are held by histologists. Hennequy ('98), Lenhossék ('98), Zimmerman ('98, in the uterus), Heidenhain ('99), Fuchs ('02, '04), Joseph ('03, '04) and others say that ciliated cells lack centrosomes; many investigators (Studnička '99, Fischel '00, Eismond '00, Henry '00, Benda '00-'01, Gurwitsch '00, '01, Ach '02, Wallengren '05, Ikeda '06, Erhard '10, and Tschassownikow '13), on the contrary, believe that they have found centrosomes in ciliated cells.

It is a difficult matter to find centrosomes with certainty; for they are minute corpuscles and often are apt to escape observation. Hence, it can hardly be said that they are absent when they cannot be detected; on the other hand, there are many other corpuscles which are similar to the centrosome in shape, position, and staining reaction, but of a different nature. Wallengren ('05) noticed such granules in ciliated cells, and said that they must be passed out of the nucleus, and to be closely related to the secreting process. We can only speak of centrosomes where the corpuscles show constant shape position and, staining powers and directly conduct mitotic division of the cell. It is very difficult, though not impossible, to follow such processes. Wallengren ('05) has already noticed that diplosome-like granules which are present between the basal corpuscles become centrosomes directing the karyokinetic cell-division.

I have noticed what are to be regarded as centrosomes in the following situations. Before describing my observations, it must be remarked that the centrosomes, in my conception, are to be recognized from the constancy of their shape, position and staining reactions, and, that whether or not these corpuscles, in reality, conduct the mitotic division, in other words, whether all



the ciliated cells which possess centrosomes always multiply by means of mitotic decision, is another matter, which will be dealt with separately in the next section.

1. *The intestines of Helix and Anodonta.* The ciliated cell from these organs contains a well-marked centrosome (figs. 6, 14, 15) which is commonly situated between the nucleus and the cuticular border, either near the cell-axis or at the periphery of the cell (fig. 6). It generally consists of two granules; the line of connection between them being in most cases parallel to the cell axis. They are surrounded with a clear halo, into which chondriocentes never enter. In the ciliated cells of the typhlosole of *Anodonta*, Erhard ('10) claimed to have found a centrosome in the form of double granules immediately below the cuticle, but the corpuscles in his figures 1, 11, and 12 are too indistinct to be distinguished from the basal corpuscles in this region.

2. *The pharynx of Hynobius larvae.* Here the centrosome of the ciliated cell is a double granule (fig. 55) between the nucleus and the cuticle, especially near the latter and often near the periphery of the cell. The granules are either equal or unequal in size, and are surrounded by a halo. The line of connection between them is usually vertical, but an oblique or even a horizontal direction is not infrequent. I have also noted occasionally a centrosome containing one, three or four granules. There are descriptions of the central corpuscle of the ciliated cell in the pharynx of *Urodela*, by Studnička ('99), Eismond ('00), Fischel ('00), Gurwitsch ('01) and Tschassownikow ('13). The first three authors state that the centrosome occurs in the form of a diplosome between the nucleus and the cuticular border, just as I have found it. Studnička, in addition, noticed, in the pharynx of *Salamandra*, a centrosome consisting of three granules. On the other hand, Gurwitsch figures a centrosome in the superficial layer of a ciliated cell from the region intermediate between the pharynx and the oesophagus of *Salamandra*; an observation with which those of the above authors and of mine are not in accord.

3. *The pharynx of Bufo.* The centrosome is situated above and near the nucleus (fig. 49, the cell to the left), and consists of

two granules, which are usually arranged in a horizontal plane. Cases in which they are separated from each other or in which they seem to consist of a single granule are not rare.

4. *The gall-duct of Rana esculenta.* The centrosome (fig. 57) surrounded by a clear halo is situated above the nucleus and consists of two granules, the axis of which either corresponds to the cell-axis or is more or less oblique to it.

5. *The trachea of Trigonocephalus and the rat.* The centrosome in both these forms, is situated between the nucleus and the cuticle, surrounded by a halo, just as described under 4 (fig. 69).

6. *The efferent tubule of testis of the mouse and rat.* The existence of centrosomes in ciliated cells in the vas efferens was noted by Zimmerman ('98), Henry ('00), Ach ('02), Ikeda ('06) and Erhard ('10). According to Zimmerman, who has investigated ciliated cells of the human epididymis, the centrosome, surrounded by a clear halo, is situated immediately below the surface, and consists of two granules, the line of connection between them being either vertical or more or less oblique to the cell-axis. The observations of Ikeda and Erhard are in accord with those of Zimmerman. On the other hand, there is some difference of opinion as to the shape and position of the centrosome. In the epididymis of man, Henry found it above and near the nucleus, while Ach says that it consists of one, two or three granules, surrounded by a clear halo and that it lies either above or by the side of the nucleus or near the cell-basis.

In ciliated cells of the efferent tubule of the mouse and rat, I have also frequently seen centrosomes, which differ considerably in shape in the two species.

In the mouse, the centrosome of the ciliated cell (figs. 82, 86) is situated immediately below the row of basal corpuscles, either in the middle or near the periphery of the cell, and consists of two granules which are always arranged in the horizontal plane. In order to distinguish them from the adjacent basal corpuscles with certainty, it is necessary to examine sections carried through the ciliated cell in an exactly vertical direction. Furthermore, I have often noticed, at the surface of neighboring cells with brush borders, a two-granulated centrosome, arranged

in the vertical direction; the upper granule often bears a cilium, thus forming the so-called 'Zentralgeisselzelle' of Zimmerman. This is found not only in the efferent tubule of the mouse, but also in that of the rat (fig. 89).

In the efferent tubule of the rat, on the contrary, there are no such typical centrosomes as above described, but instead curious ring-shaped corpuscles in the transparent zone below the cuticle (figs. 87, 88). Most of these rings have a smooth internal and an uneven outer edge; in a certain sense they can be spoken of as being formed by the apposition of granules. Such rings are not always single, but there are many cells with two, either of equal or of unequal size (fig. 87); in either case they are arranged in a horizontal direction and connected with each other by a fiber.

I am inclined to believe, from the position and staining of these rings, that they are derived from the centrosomes; a centrosome divides repeatedly and forms a ring by secondary fusion of separated particles; the connecting fiber representing the so-called 'centrodesmosis.' And, as a form of transition I have found a cell with a ring and a granule, of which the latter can be regarded as a component, still not divided, of the centrosome. Whether the 'Zentralkörperballen' found by Benda ('00) and Ikeda ('06) in the efferent tubule of the human testis and my ring are of the same character or not remains uncertain, but there are some differences between the two concerning the shape and position. Moreover, these authors admit that the 'Zentralkörperballen' take part in the formation of the cilia, a view with which I do not agree, for, on the one hand, the ciliated cell with such rings is always in the fully developed state and, on the other hand, it seems conclusive that the ciliary apparatus never originates from the centrosome, but from a certain other constituent of the cell, as will be afterwards described.

As above described, Henneguy ('98), Lenhossék ('98), Joseph ('03) and Fuchs ('02, '04) believe that, in consequence of the absence of the centrosome, mitotic division does not occur in the ciliated cell. Ach ('02) and Tschassnowikow ('13) say that no mitotic figure occurs in ciliated cells in spite of the existence

of the centrosome; Tschassownikow especially points out that the ciliated cell, though it shows no karyokinetic figure, can scarcely be said to be incapable of undergoing division, for it is provided with organs of cell-division, i.e., the nucleus and the centrosome. On the other hand, many of the investigators (Hammer '97, Gurwitsch '01, Maier '03, Wallengren '05, Erhard '10, '11, Gutheil '11) believe they have found mitotic figures in ciliated cells. Gurwitsch represents in his figure 19, a cell with a nucleus at the spireme-stage, which he takes as a ciliated cell; judging from his figure, however, it seems to me that the cell has no cilia and basal corpuscles, but a relatively high cuticular border. Just as little is certain whether the mitotic figures found by Maier in the epithelium of the gills of Triton and by Erhard in ependyma cells of an *Acanthias* embryo belong to ciliated or non-ciliated cells. On the contrary, the observation of Erhard in the typhlosoles, and especially that of Wallengren on gills of *Anodonta* appears to me to admit of very little doubt.

In the intestines of *Anodonta* and *Helix* and in the gall duct of *Helix* I have been able to follow the karyokinetic process of the ciliated cell, from the prophase to the anaphase. In the following I deal with the intestine of *Anodonta* as a representative of such cells.

*Prophase* (figs. 17, 18). The first change of the nucleus consists, as usual, in the formation of the spireme; the cytoplasm becomes clear and the chondriocentes, which gradually grow pale, are scattered over the whole of the cytoplasm. At first the basal corpuscles still stain deeply, but afterwards they disappear. Contrary to the view of Wallengren ('05) I have found that the cilium and the basal corpuscle become lost simultaneously. All of the cilia and the basal corpuscles, however, do not disappear suddenly, but by degrees, so that it often occurs that a few basal corpuscles with cilia are left behind for some time (fig. 19); moreover I have noticed, in the intestine of *Helix*, cells with distinct cilia, even in the metaphase. The ciliated cell in the resting condition is long and narrow in shape; but on entering upon the karyokinetic process it becomes swollen where the



nucleus is situated, so that it takes the form of a pear. Although the distal border of the cell in question becomes diminished in extent, I have never been able to find that it becomes rounded off, after separating from the surface of the epithelium, as described by Wallengren. In some cases the distal border is either bulged (fig. 19) or indented, in consequence of the pressure of neighboring cells. The cuticular border, so far as can be seen from our materials, is left intact throughout the process of cell-division. Since the basal corpuscles are lost to view, the limits between the cytoplasm and the cuticle become indistinct, so that it looks as if the cuticle had disappeared, as Wallengren asserts.

*Metaphase (fig. 19).* The plane of division, so far as could be observed, is at right angles to the surface of the epithelium, so that the two resulting daughter-cells lie side by side.

*Anaphase (fig. 20).* The chromosomes send off lateral branches which join or anastomose together in a reticular manner while the nuclear membrane and nucleolus reappear. The cytoplasmic fission sets in at the inferior end of the cell and gradually proceeds upwards. Near and below the cuticle there appear the so-called intermediate corpuscle, from which protoplasmic filaments run towards the centrosomes. The chondriocentes, again becoming deeply stained, are arranged in the vertical direction, and are chiefly accumulated below the cuticle; the cell becomes in the meanwhile lengthened out downwards. Next, basal corpuscles and cilia make their appearance, which process will be referred to later on.

Although I have subjected various epithelial tissues of vertebrates to a careful examination, I have never been able to find karyokinetic division of the ciliated cell; the mitotic figures which are often seen in the ciliated epithelium, do not belong to ciliated cells, but either to non-ciliated columnar, or to basal cells or to glandular cells.

### *Summary*

1. The centrosome can be detected in ciliated cells of both invertebrates and vertebrates.



2. It can not be said that the centrosome is always lacking in case it can not be detected in the ciliated cell.
3. The centrosome may be situated in every level between the nucleus and the cuticle.
4. I have been able to find mitosis of the ciliated cells in invertebrates only.

#### C. AMITOSIS OF CILIATED CELLS

Henry ('99, '00), Ach ('02), Wallengren ('05), Jordan ('13) and others have noticed ciliated cells with two nuclei, which, according to these authors must be a result of direct nuclear division. I also found what appears to be amitosis of the ciliated cell in the following places.

1. *The intestine and hepatic duct of Helix.* Here often occur ciliated cells with two nuclei (figs. 13, 14) which are oval in shape; these cells also may contain, as usual, a centrosome consisting of two granules, situated about midway between the cuticle and the nucleus (fig. 14). On close examination of preparations it would appear that these two nuclei are produced by direct nuclear division. In the middle of the nucleus there first appears a transverse furrow (fig. 12); by the deepening of which it becomes separated into two nearly equal parts. I have never observed cases in which the division was effected by the stretching of the nucleus. Cell-division follows nuclear division; the cell-boundary appears between the two nuclei; thus two superposed daughter-cells are produced, of which the upper alone bears cilia, while the lower one becomes transformed into a basal cell and then, by the accumulation of secreting granules in its interior, into a glandular cell, which elongates upwards between the ciliated cells until it reaches the surface of the epithelium.

Besides cells with two nuclei, there are occasionally seen those with three, four or five nuclei; even in these cases the internal structure at first shows no noticeable change. Whether these multinucleated cells are capable of division is doubtful; in all likelihood one or several of the nuclei undergo degeneration later on.

2. *The pharynx of Amphibia (Rana temporaria and esculenta, Bufo, Triton).* In the normal condition, the surface of the

nuclei of the ciliated pharyngeal cells is smooth or shows at the most some irregularities. At the beginning of the nuclear constriction the nuclear membrane is characteristically thrown into folds, this is most marked in *Rana temporaria*. The cleavage-plane of the nucleus is either vertical (figs. 42, 49) or oblique, or even horizontal (fig. 44). The furrow formed by a pushing in of the nuclear membrane appears, in *Rana esculenta*, as two parallel straight lines (fig. 42), but in *Rana temporaria* as zigzag lines which can be made out only by careful focussing (fig. 51). Between the structure of the amitotic dividing nucleus and that of the normal one there are no noteworthy differences, as will be seen in my figures. The daughter nuclei are at first closely apposed by their divided faces; later on, they are gradually separated from each other (figs. 43, 52). I have observed in *Bufo* a curious phenomenon in the behavior of the centrosome in the amitotic process: the ciliated cell in the pharyngeal epithelium of this animal, as already mentioned, contains a bigranulated centrosome lying above the nucleus (fig. 49, the cell to the left). With the separation of the dividing nuclei from each other, each of the granules accompanies each daughter-nucleus (fig. 49, the cell to the right), a fact which indicates that the centrosome is not entirely independent of amitotic nuclear division.

Cell-division follows nuclear division. Since the cytoplasmic fission always takes place along its longer axis of the cell, being inaugurated either at the upper end (fig. 45) or at the lower (fig. 48), it must be thought that the superposed nuclei, as it often occurs, undergo locomotion before the cell-division sets in. In the amitotic process there are no visible structural changes of the cytoplasm, the nucleus, or of the ciliary apparatus, except that the two former increase more or less in volume.

3. *The gall-duct of Rana temporaria.* Ciliated cells with two closely applied nuclei occur, though not frequently, in the bile-duct epithelium. The process of cell-cleavage, however, was not observed.

4. *The oviduct of Rana temporaria and esculenta.* In the oviducal epithelium are large ciliated cells with two nuclei (fig. 64), which cannot be interpreted except as having been pro-

duced by amitosis, for mitosis of ciliated cells has never been observed here. Next, cytoplasmic division follows, the plane of which is always vertical. Sometimes the amitotic process seems to occur repeatedly; in figure 63 is a ciliated cell with six nuclei which are closely apposed on their flat side-faces; I am of opinion that the nucleus, in this case, has repeatedly undergone amitotic division, while cytoplasmic fission has not begun.

5. *Efferent tubules of Reptilia.* Ciliated cells with two nuclei often occur in the efferent tubules of *Trigonocephalus* and *Elaphis* (figs. 72-74), but rarely in the lizard. The direction of constriction of the nuclear membrane is not a constant; the plane of cytoplasmic division, however, is always perpendicular.

6. *The oviduct of the lizard.* Binucleated ciliated cells are rarely seen here (fig. 77); the two nuclei which are evident must have been produced by amitosis, for mitosis has not been detected.

7. *The trachea of Trigonocephalus.* The two nuclei, either superposed or juxtaposed, are closely apposed by their flat faces; between these extremes there occur intermediate conditions.

8. *The trachea of the rat.* The arrangement of the two nuclei is the same as described under 7 (fig. 93). The cell-body gradually enlarges, especially increases in width. The cleavage of the cytoplasm begins either at the upper or at the lower end of the cell (fig. 94), and passes perpendicularly between the nuclei. I have also often noticed that a granule accompanies each of the divided nuclei; from their position, shape and staining it is probable that these granules are derived from the pre-existing centrosome.

### Summary

1. The ciliated cell may divide by amitosis.
2. This cell multiplication by amitosis occurs only in vertebrates.
3. The nucleus and the cell-body are constricted by pushing in of the nuclear membrane.
4. Cell-division follows nuclear division.
5. The ciliary apparatus remains unaltered in the amitotic process.

6. It seems probable that there exists some connection between the centrosome and the amitosis.

#### D. SIGNIFICANCE OF MITOSIS AND AMITOSIS OF CILIATED CELLS

As mentioned above, there are two methods of division of the ciliated cell, mitotic and amitotic. Mitosis occurs only in invertebrates; in vertebrates I have never been able to find it. Those who accept the hypothesis of Henne-guy and Lenhossék, assert that the absence of mitosis in ciliated cells is a consequence of the lack of the centrosome. I do not agree with this, for the centrosome can easily be detected in ciliated cells in which mitosis does not occur. On the contrary, amitosis is the sole method of division of ciliated cells of vertebrates, in spite of the presence of the centrosome.

As to the significance of amitosis, two distinct and opposed views are held by histologists. According to one view, urged strongly by Flemming, Ziegler, and vom Rath, amitosis is not accompanied by the cytoplasmic division, but such a cell degenerates sooner or later. That the nuclei of degenerating cells may multiply by amitosis has received much attention (Nissen '86, Heidenhain '90, Plate '98, Dobell '07, Reichenow '08); and, in fact, I have also noticed that the nucleus multiplies by direct division, in the degeneration of certain glandular cells in the larval epidermis of some Amphibia (Saguchi '15).

On the other hand, Child ('07), Patterson ('08), Maximow ('08), Des Cilleules ('14) and others affirm that amitosis is not always degenerative, but can be accompanied by the actual cell-multiplication; and, that mitosis follows amitosis and vice versa. According to Child and Patterson, amitosis is in close relation to the rapid nuclear multiplication and accordingly to the growth of tissues. Child, in addition, remarks as to the occurrence of amitosis as follows: "Moreover, in several cases I have noted that in growing tissues where nuclei of different size are present, mitosis seems to occur more frequently in larger nuclei surrounded by considerable undifferentiated cytoplasm, while amitosis is more characteristic of the smaller nuclei with scanty cytoplasm." Recently, Jordan ('13) described ciliated cells

with two nuclei in the epididymis of various vertebrate animals, in the trachea of the cat, in gills of *Unio*, and he believes that these nuclei are produced by amitosis which is connected with the following cytoplasmic division. He expresses himself regarding the cause of amitosis in ciliated cells as follows: "The fundamental cause of amitotic cell division in ciliated cells is the destruction of the centrosome in the formation of the basal bodies from which the cilia develop." Contrary to the view of this author, Henry ('00), Ach ('02), Ikeda ('06), and I have noted the existence of centrosomes in ciliated cells; and, on the other hand, there are many cases in which they may be detected in cells with two nuclei resulting from amitosis (Flemming, Maximow).

My conclusions concerning the significance of mitosis and amitosis in ciliated cells may be summarized as follows:

1. The occurrence of amitosis in ciliated cells is not owing to the lack of the centrosome; for the latter can be detected in many cases in such cells.

2. The sole method of multiplication of ciliated cells in invertebrates is by mitosis, in vertebrates by amitosis; the cause bringing about this difference between these subkingdoms, must be due essentially to the degree of differentiation of the cell-plasm.

## VII. DEVELOPMENT OF CILIATED CELLS

### A. HISTORICAL

As early as 1875 Eichhorst described the development of cilia in the ependyma cells of the spinal cord of man; a transparent cuticle first appears at the distal border of the columnar cell; this afterwards becomes striated longitudinally, the cilia passing through this striated cuticle. According to Engelmann ('80), cilia regenerate by the elongation of their rootlets, while Fol ('96) described the formation of cilia by the prolongation of the distal cell-border. Gurwitsch ('01) distinguishes two types of ciliogenesis: (1) first, the basal corpuscles appear at the nodes of the alveolar meshes of the cuticle and afterwards the cilia become developed from them (in the oviduct of the rabbit and



in the pharynx of Bufo); (2) first, the alveolar septa themselves become transformed into the cilia, while the basal corpuscles secondarily make their appearance at their basis (in the pharynx of Salamandra larvae). On the other hand, the observations of Benda ('00), Fuchs ('04) and Ikeda ('06) on the efferent tubules of the human testis, had a marked influence upon the development of the so-called Henneguy-Lenhossék's hypothesis. According to these authors the centrosome, after multiplying by repeated divisions, gives rise to the basal corpuscles, from which the cilia develop. Wallengren ('05) studied ciliogenesis in the daughter cells produced by the mitotic division of the ciliated cell and found that, contrary to this view, the basal corpuscles are developed, independently of the centrosome, within the superficial portion of the cell-protoplasm, a view with which those of Heidenhain ('99) and Erhard ('10) are in accord. Ach ('02) asserts that the basal corpuscles are derived from granules situated above the nucleus, from which they are cast off, while Gutheil ('11) maintains that the ciliary apparatus is developed from the microsomes suspended on protoplasmic networks.

#### B. EMBRYONIC DEVELOPMENT OF THE CILIARY APPARATUS

1. *The epidermis of Amphibian larvae (Rhacophorus, Hynobius and Hyla).* The development of the ciliated cell in the epidermis of these larvae begins at an early embryonic period in which the larvae are still enclosed within the gelatinous coat. The epithelial cell which is preparing to transform into ciliated (fig. 23, the cell to the left) is always provided with a well-marked cuticular border, the actual structure of which has been already referred to above in the section "Relations between the cilia and the crust or cuticle." The cells in question are most commonly large, so that the lower ends often reach the basement membrane; most of these cells are laden with yolk spherules of various size (figs. 24, 26), in the intervals between which mitochondrial filaments (chondriocentes) course in different directions; they are also gathered in considerable numbers below the cuticle. At successive periods, these chondriocentes, after or without becoming vertically arranged, pass into the cuticle (fig. 24).

In this they do not pass into the alveoli, but ascend along the striae, which are nothing but nodes of the alveolar walls in the cuticle. Soon all the striae are occupied by the immigrant chondriocotes, so that deeply staining parallel striae occupy the same place as the alveolar ones (fig. 25). These, however, are not always parallel to the radii, sometimes they are more or less inclined in one direction, as seen in figure 25. This is, in all probability, due to the previous inclination of the alveolar walls. At successive periods the mitochondrial striae gradually grow pale; there are, however, cases in which it occurs only after completion of the development of the cilia. Next, they emit from their distal ends minute faintly stained prolongations (fig. 26), which are the young cilia. At the beginning they are relatively thick and short; later, they lengthen out (fig. 27). The chondriocotes are not used up in the formation of the cilia, but a number of them remain behind in the cell, especially gathered below the cuticle. From this manner of development it is possible that some of these chondriocotes are in continuity with the cilia, and give rise to the so-called rootlets.

2. *The pharynx of Rhacophorus larvae.* The ciliogenesis begins in a larva about 15 mm. in length. The chondriocotes, growing more and more pale, ascend along the cuticular striae and project beyond the limits of the cuticle (fig. 56). At first they are short, and often curved in the shape of hooks, but afterwards they lengthen out.

3. *Tela chorioidea of Amphibian larvae (Hyla, Rhacophorus and Hynobius).* In the early embryonic stage, the tela epithelium of *Hyla* consists of flattened cells, which contain numerous yolk-spherules and chondriocotes; the latter are accumulated near the upper border of the cell (fig. 34). In the next stage the cilia make their appearance (fig. 35). From the fact that the cilia are produced in that part which coincides with the accumulation of chondriocotes, the inference is warranted that there may be a genetic connection between the two.

4. *The ciliated infundibulum of Hyla larvae.* The chondriocotes are collected below the cuticle through which they pass out of the cell in order to form the cilia; the continuity between the two is readily discernible in favorable conditions (fig. 40).

Although, from these observations no definite conclusion as regards the development of the basal corpuscles and the rootlets, can be reached there is no indication of the formation of cilia from pre-existing basal corpuscles. The chondriocotes give rise to the cilia. The basal corpuscles and the rootlets are, in all probability, formed by the special differentiation of the chondriocotes remaining behind in the cell.

C. DEVELOPMENT OF CILIATED CELLS IN THE EFFERENT TUBULE  
OF TESTIS OF THE MOUSE AND RAT

Contrary to the view of Hammer ('97), Henry ('99), Ach ('02) and Jordan ('13), I could not find any division figure, either mitotic or amitotic, in ciliated cells of the efferent tubules; the regeneration of these cells is, rather, effected, as mentioned before, by the transformation of cells with brush borders; the ciliogenesis which occurs in this corresponds in all respects with that of the embryonal stage; first, the chondriocotes increase largely in number and are chiefly accumulated between the nucleus and the distal cell-border (fig. 79). It is certain that they are not derived from the centrosome, though nothing is known of the manner of their increase. They then proceed towards the distal cell-border and transform into rod-like corpuscles arranged in linear rows (fig. 80). These rods can scarcely be said to be basal corpuscles, for they stain in the same way as the chondriocotes. They emit, at successive periods, short initial cilia (fig. 81), which gradually lengthen (fig. 82). In figure 82 there is, as usual, a centrosome consisting of two granules, the existence of which indicates that the latter takes no part in the formation of the cilia. That the process of ciliogenesis takes place in cells with brush borders has been already referred to; the developing cilia are seen to pass, not through the interspace between the hairs of the brush border, but through their axes, as represented in figure 84 (the cell to the right). Since the cilia are longer than the hairs, it is evident that the former project beyond the distal extremity of the latter, which can no longer be detected in fully developed ciliated cells.

Whether or not the ring-shaped corpuscles which I found in ciliated cells of the efferent tubule of the rat, are the same as the 'Zentralkörperballen' of Benda and Ikeda, I am not certain; I have not, however, sufficient evidence to show that they have any connection with ciliogenesis. It seems probable that these structures, as well as the granules or threads described by Fuchs, are similar to the chondriocentes.

#### D. CILIOGENESIS IN THE DAUGHTER-CELLS PRODUCED BY MITOSIS OF THE CILIATED CELL

As already mentioned, the ciliated cells in the intestine of *Anodonta* multiply by mitosis; at the beginning of the metaphase the basal corpuscles and the cilia disappear, and in the anaphase the ciliogenesis sets in; the process is, in the main features, similar to that of embryonic development of the cilia. The chondriocentes now give the previous staining reaction and are collected above the nucleus (fig. 21). At successive periods, some of these filaments proceed towards the cuticle, their upper ends often swelling out into bulbous enlargements, from which the cilia pass out through the cuticle (fig. 22). These granules are, in all probability, not identical with basal corpuscles, but mere local accumulations, of the mitochondrial substance.

#### *Summary*

1. The ciliary apparatus is produced by the differentiation of mitochondria or chondriocentes, whether the process occurs in embryonic or in adult cells.
2. The centrosome takes no part in the formation of the cilia.

#### VIII. HENNEGUY-LENHOSSÉK'S HYPOTHESIS

Heneguy and Lenhossék, at nearly the same time ('98), but independently, formulated an hypothesis that the ciliary apparatus, especially the basal corpuscles are derived from the centrosome; an hypothesis which has since obtained some adherence among histologists (Benda '00, Fürst '00, Holmgren '03, Fuchs '04, Joseph '05, Ikeda '06). The arguments in favor of this

hypothesis are: (a) the shape and position of basal corpuscles in the ciliated cell correspond with those of the centrosome in neighboring non-ciliated cells (Lenhossék, Holmgren); (b) viewed in unstained preparations, the basal corpuscle refracts light as strongly as does the centrosome (Lenhossék); (c) both the above structures show stain in the same way (Lenhossék); (d) the ciliated cell lacks centrosomes (Lenhossék, Füss, Fuchs, Jordan); (e) the ciliated cell bears resemblance either to a spermatid (Henneguy, Lenhossék) or to a 'Zentralgeisselzelle' (Joseph); (f) no mitotic figure is seen in the ciliated cell, because the latter lacks centrosomes (Henneguy, Lenhossék, Fuchs, Joseph); (g) the basal corpuscles arise from the centrosome.

There are many other investigators whose opinions are adverse to the above hypothesis; they bring forward the following facts in support of their view; (a) the granules which stain black with iron-haematoxylin cannot always be said to be centrosomes, for there are many other cell-constituents which give the same reaction (Studnička '99, Fischer '99); (b) contrary to the view of Lenhossék, the centrosome is often situated deeply; (c) it can scarcely be said that the ciliated cell lacks centrosomes when it is not met with (Merkel '08); (d) the ciliated cell has a centrosome (Studnička '99, Eismond '00, Fischel '00, Henry '00, Gurwitsch '00, Wallengren '05, Erhard '10, Tschassownikow '13); (e) sometimes mitosis is observed in ciliated cells (Gurwitsch '00, '01, Maier '03, Wallengren '05, Erhard '10, Gutheil '11); (f) the basal corpuscles are not derived from the centrosome, but from other cell-constituents (Gurwitsch '00, '01, Wallengren '05, Erhard '10, Gutheil '11).

My observations also are not in accordance with the hypothesis of Henneguy and Lenhossék; they are summarized as follows:

1. The basal corpuscle refracts light strongly and stains deeply with iron-haematoxylin, but these properties are not characteristic of these bodies.

2. I have found centrosomes in many ciliated cells.

3. The existence of the centrosome does not always signify the occurrence of mitosis, for the ciliated cells of vertebrates do not multiply by mitosis, though they contain distinct centrosomes.



4. The resemblance of ciliated cells to spermatids or to 'Zentralgeisselzellen' must be regarded as accidental.

5. The ciliary apparatus, especially the cilia, are not derived from the centrosome, but from the chondriocentes.

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#### EXPLANATION OF PLATES

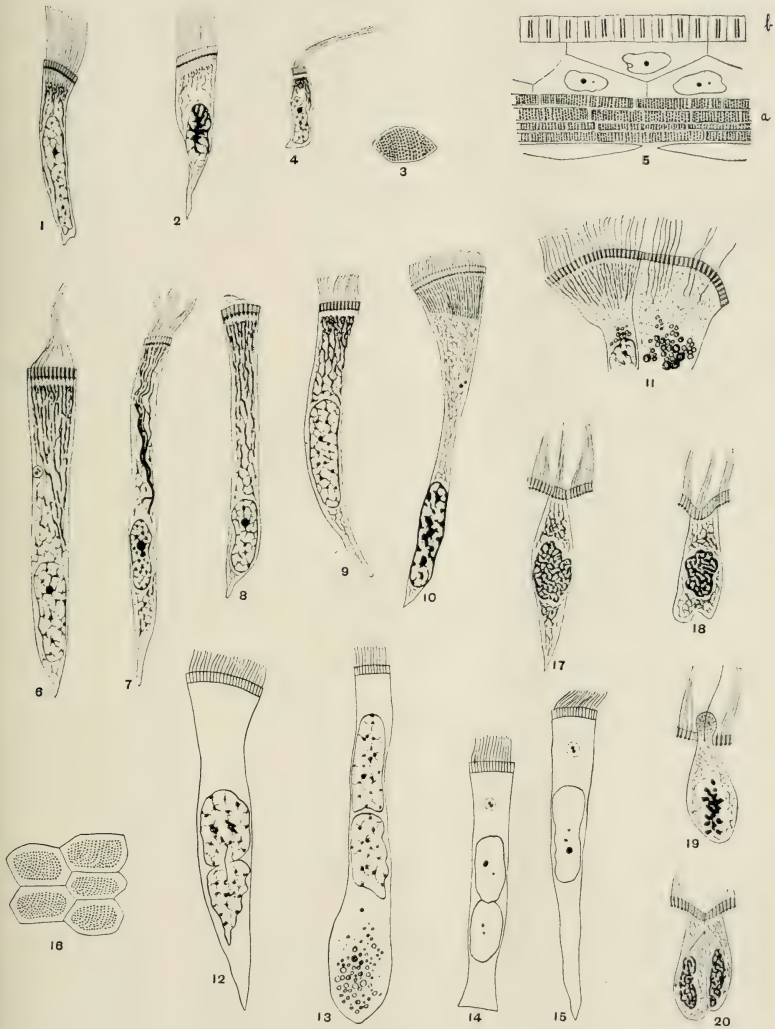
All figures are drawn with the Zeiss camera under Zeiss  $\frac{1}{1\frac{1}{2}}$  oil-immersion objective and Zeiss compensating ocular 18, at the distance of 250 mm. tube length 160 mm. By reproduction they were reduced to three-fourths of the original. Figures 2, 3, 5, 7, 10, 14, 15, 30-33, 37-39, 46, 47, 49, 50, 55, 57, 69, 67, 73, 79-83, 86, 90, 92, 95 are from preparations stained by iron-haematoxylin, after fixation with sublimate; the others from Meves preparations.

#### PLATE 1

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- 9, 13 to 15 From the intestine of Helix.
- 10 to 12 From the hepatic duct of Helix.





## PLATE 2

### EXPLANATION OF FIGURES

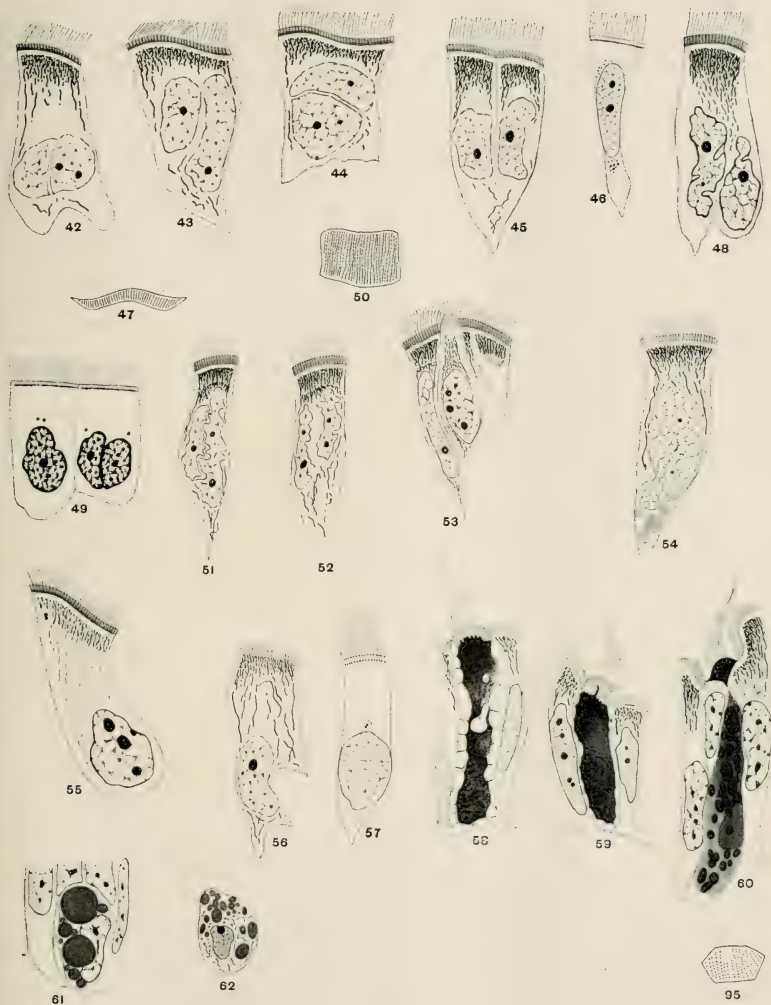
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- 23 From the epidermis of the tail of 6 mm. *Hyla* larva.
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# THE PRIMORDIAL CRANIUM OF THE CAT

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THIRTY FIGURES (TWELVE PLATES)

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## INTRODUCTION

Studies of the primordial cranium of mammals have in most instances dealt with but a single stage, and offer, therefore, almost no data on developmental processes. Moreover, there seems to be a tendency, unfortunately, to pursue the study after this manner and so to continue limiting inquiry to the methods of comparative, descriptive anatomy. This criticism cannot be made of investigation of the crania of the ichthyopsida. It has been rather the rule than the exception for research in this group to include as complete a survey of developmental processes as the technique of the time permitted; that is, the studies proceeded toward the solution of cranial problems by comparative embryological methods. No doubt, certain physical conditions have in the one case been an obstacle to embryological work. The difficulty of securing material in control and of sufficient amount, and the great extent to which the investigation of even a few stages leads one, are factors which have determined in no small degree the methods and results of research on the crania of higher forms. However, these difficulties are not serious and should not stand in the way of gaining for the mam-



malian cranium that knowledge of its development which is so much desired.

For the study of problems of the mammalian primordial cranium, the domestic cat offers material possessing certain advantages. In general, the processes of chondrification proceed sufficiently slowly to permit of easy determination of the origin of parts and of subsequent study of their individual histories. Also, the cat among the domestic mammals has remained standardized to a degree not exceeded, if equalled, by any other, and is on this account of value for anatomical study. Yet, notwithstanding these points in its favor, the house cat has not been utilized for cranial study to the extent that might be anticipated. There is at the present time no paper on the development of the cranium as a whole and no extensive description of a single stage in the course of its development. Not even Parker described the primordial cranium of the domestic cat. The only investigation which attempts to deal with the whole chondrocranium is that by Decker in which brief accounts of two rather advanced stages are presented. Wineza's communication discusses a number of questions, for the solution of which cat material has been employed, but it does not include all regions of the cranium. This valuable paper is available to a relatively small number of investigators since it was published in the Polish language.

What has been said in regard to cat for the study of the whole chondrocranium does not apply in the case of investigations of special problems, although even in the latter case this material has been used to a more limited extent than might be expected. Of the cranial regions usually recognized, the occipital in cat has received less attention than have the others. The otic region is represented by a number of papers concerned for the most part with late embryonic stages and adult conditions. Among these, the publications of Spence, Bondy, van Kampen, and Denker have been consulted in the present work. Wineza's paper deals especially with the ala temporalis and carotid foramen; an article by Arai includes the cat in a study of the craniopharyngeal canal, and a paper by Williams deals

with the notochord in its later history. In the ethmoidal region interest has been centered upon the skeletal structure of the floor of the nose. Harvey, Broom, and Zuckerkandl have each contributed valuable descriptions of the cartilaginous framework about Jacobson's organ and the incisive ducts in cat.

In the present paper the monographs on the anatomy of cat by Strauss-Durekheim, Wilder and Gage, Jayne, and Reighard have been very helpful, as have those works of a more general scope, but including the cat, by Chauveau, Paul Martin, and Weber. Finally should be mentioned those studies, in which cat has offered the material, of head structures other than the cranium which are inseparably connected with the problems of the skeleton, many of which have aided the present investigation. Among these are, specially, Retzius' researches on the ear; Göppert and Corning on the ocular muscles; Peter, Born and Seydel on the nose; Froriep and van Wijhe on the occipito-spinal nerves.

This paper is concerned with some of the problems of cranial development in mammals, the chondrocranium (exclusive of the visceral skeleton) in cat serving as the material for study. It was the intention to give the descriptive portions of the several cranial regions, as far as possible equal treatment, but to limit the discussion to a few problems upon which some light was thrown by the structure and development of the cranium in the particular form under consideration. The occipital region was looked to with keen interest as an unworked field for observation, and for any evidence of those processes revealed by Weiss, Gaupp, and Weigner in mammals which indicate a close resemblance to, if not an actual repetition of the steps in the development of the atlas. As stated above, the work on the otic region, hitherto, treats of late embryonic and adult conditions. In the present paper, attention has been directed mainly to conditions of early development, such as the origin of the cartilaginous otic capsule, the comparison of the embryonic vestibule with that of reptiles and the theoretical questions concerned with the development of the cochlear skeleton. Consideration of questions relating to the development and signifi-

cance of the ala temporalis and to the formation of the hypophyseal skeleton forms the main part of the study of orbito-temporal region. Concerning the origin and early history of the ethmoidal skeleton of mammals comparatively little is known, whereas, on the contrary, a great literature exists on the development of the nasal conchae, floor of the nose and nasal sinuses. Cat material was found favorable for the investigation of many early processes of ethmoidal development, and to a discussion of these, practically the whole of the section on the ethmoidal region has been devoted.

#### MATERIAL AND METHODS

The present study starts with a cat embryo of 23.1 mm., series no. 466 of the Harvard Embryological Collection. From this, a model of the chondrocranium was reconstructed by students of the Harvard Medical School under the direction of the author while a teaching fellow in histology in that institution.<sup>1</sup> The model, enlarged thirty-three times, includes, in addition to the chondrocranium, the ossifications, main bloodvessels and nerves. It is an example of care and industry and as such has been on the one hand invaluable as material for study and on the other a reminder of the enthusiasm and earnest effort of those students, then in their first year of medical study, Messrs. G. D. Cutler, I. Gerber and R. D. Leonard, who responded to the suggestion to undertake the labors of cranial reconstruction.

Through the kindness of the late Professor Minot and Professor F. T. Lewis, the model, together with the following series of cat embryos from the collection at the Harvard Medical School, was placed at my disposal in St. Louis.

<sup>1</sup> The model was reconstructed in 1907. The present investigation was undertaken in St. Louis several years later but on account of interruptions its progress was delayed. I take this opportunity to acknowledge the many courtesies of Prof. J. S. Kingsley and the staff of the Marine Biological Laboratory at South Harpswell, Maine, where during the summer of 1915 it was my privilege to work.

|                                   |            |
|-----------------------------------|------------|
| Cat 9.7 mm., frontal,             | Series 448 |
| Cat 10.6 mm., frontal,            | Series 476 |
| Cat 12.0 mm., frontal,            | Series 404 |
| Cat 12.0 mm., horizontal of head, | Series 403 |
| Cat 12.0 mm., sagittal,           | Series 400 |
| Cat 17.0 mm., sagittal,           | Series 492 |

I am indebted also to Professor Huntington for the use of series of cat embryos of the following stages, from the collection at the College of Physicians and Surgeons, Columbia University.

|             |            |
|-------------|------------|
| Cat 10 mm., | Series 500 |
| Cat 11 mm., | Series 473 |
| Cat 12 mm., | Series 263 |
| Cat 13 mm., | Series 262 |

From the collection in the Department of Anatomy of Washington University the following were studied:

|                         |           |
|-------------------------|-----------|
| Cat 12 mm., frontal,    | Series 50 |
| Cat 12 mm., transverse, | Series 52 |
| Cat 15 mm., transverse, | Series 56 |
| Cat 17 mm.,             | Series 80 |
| Cat 17 mm., sagittal,   | Series 81 |
| Cat 17 mm., transverse, | Series 82 |

Several special reconstructions were made of complex regions. One is of the left otic capsule of the 23.1 mm. embryo; a second represents the course and relations of the facial nerve in the same specimen; a third, the eye-ball and its muscles in relation to the chondrocranium; a fourth the cranial relations of the notochord. The atlas and epistropheus were included in the main model. The base of the cranium in the region of the carotid artery was reconstructed from a 12 mm. embryo; from the same a reconstruction of the hypophyseal cartilage was made. The nasal capsule and nasal sac have been modeled from an embryo of 17 mm.

The study of all stages of the chondrocranium was facilitated by a collection of preparations made by the van Wijhe ('02)

method. These are of great value for studies of the cartilaginous skeleton, especially when used in comparison with the sections. Cat embryos were fixed in formalin, stained with methylene blue, decolorized, cleared and preserved in liquid Canada balsam. The last procedure, which departs slightly from van Wijhe's final treatment, is of much advantage in permitting the free handling and turning of the specimen so that it may be easily observed from every aspect. The following stages were prepared and studied: Cat embryos of 10 mm., 12 mm., 15 mm., 17 mm., 20 mm., 24 mm., 30 mm., and 35 mm.

As stated above, the present paper centers upon a particular stage, with which earlier and later stages have been compared. In accordance with this method the structures of each region of the chondrocranium as found in the stage of 23.1 mm., that is, the stage of the model, are first described, then younger stages are considered.

#### PART I. OBSERVATIONS

##### *Basal plate.—Embryos of 23.1 mm.*

The term basal plate is used here to designate that portion of the floor of the chondrocranium corresponding in cephalocaudal extent with the cranial part of the notochord, and in lateral extent to the level of the basal foramina and cochlear capsule.

The basal plate in the embryo of 23.1 mm. is represented in figures 1, 2 and 3. In the otic region it is very narrow; in the occipital region, on the contrary, very broad. Foramina are present at the margin of the basal plate, between it and the cranial side walls, but no openings exist in the plate itself. These marginal foramina are the fissura basi-cochlearis posterior, the jugular and hypoglossal foramina.

The anterior, or otic portion of the basal plate, subcylindrical in shape, lies between the cochlear prominences, and, within



the cranium, forms the bottom of a deep sulcus whose sides are constituted by the median walls of the cochleae. This sulcus is occupied by no part of the brain, but is filled with a loose web of mesenchymal tissue, in which the basilar artery and abducent nerves run (fig. 22). Continuity between the cochlear prominence and basal plate is brought about by means of a thin strip of cartilage interrupted by the marginal foramina above mentioned. This strip is merely a septum between two oppositely placed grooves, one outside, the other within the cranium, which, in their semicircular courses, demarcate the limits between the ear capsule and cranial floor (figs. 1, 2). These grooves extend anteriorly on the outside of the cranium beyond the confines of the basal plate and include the carotid foramina; posteriorly, both within and without the cranium, they have a somewhat lateral direction between the occipital division of the floor and the otic capsule. Here are two large openings, the jugular foramen, to be presently described, and the fissura basicochlearis posterior (figs. 2, 12). The latter is sickle-shaped, broad laterally where it approaches the jugular foramen, from which it is separated by the commissura basivestibularis. It gives passage to no nerve or large vessel.

The occipital portion of the basal plate forms the floor of the occipital region of the cranium. It presents a slightly concave intracranial surface and a convex face directed toward the nasopharyngeal duct; at the sides it is continuous, at the level of the hypoglossal canals and jugular foramina, with the lateral occipital walls. The caudal free margin is concave from side to side, forming the ventral margin of the foramen occipitale magnum, where on each side is the basal, smaller part of the occipital condyle (fig. 19); between these is a shallow incisura occipitalis anterior.

The following observations were made on the position, relations and termination of the cranial portion of the notochord. As shown in figures 14 and 19, this structure passes through the middle of the body of the epistropheus, inclines ventrally on entering the dens, and holds a position ventral of the middle of the dens throughout the rest of its vertebral course. It leaves

the dens from the ventral surface, not at its extremity, and enters the ligamentum apicis dentis. It then passes over into the cranium, lying at first just beneath the perichondrium of the dorsal surface of the cranial floor, with the great thickness of the caudal edge of the basal plate beneath it. From this point the chorda dips into the cartilage of the floor as it extends forward, lying midway between the cranial and pharyngeal surfaces in the middle third of the plate. At no point does it sink nearer the pharyngeal surface nor was there observed any trace of a connection with the pharyngeal wall. Proceeding toward the sella turcica, the notochord gradually approaches the intracranial surface of the basal plate, until it attains the level of the dorsum sellae where it makes a rather abrupt bend so as to come to lie beneath the perichondrium of the caudal surface of the back of the saddle. Its terminal piece is marked by distortion to some extent and by a few irregular turns. Although shrunk in many places, and showing other evidences of degeneration, the head notochord is, nevertheless, continuous throughout. Within the apical ligament it is considerably expanded.

#### *Occipital region*

From the basal plate, the side walls of the occipital region, the lateral occipital arches, continue laterally and dorsally, becoming confluent with the otic capsule, parietal plate and tectum posterius (figs. 1, 2, 3, 4). They lie in a plane transverse to the longitudinal axis of the posterior half of the cranium, but, owing to the flexure of the long axis of the whole cranium, this is at the same time, parallel with the plane of the floor of the nose.

The lateral occipital arches are connected with the pars canalicularis of the otic capsule by synchondrosis, marked in the sections by a narrow plane of young cartilage extending a considerable distance from the jugular foramen toward the parietal plate. Within the cranium a deep, wide groove, the sulcus sigmoideus, lodging the transverse sinus, lies opposite the ventral portion of the synchondrosis and leads to the jugular

foramen. Outside the wall a corresponding groove separates the paracondyloid process (figs. 2, 3) from the pars canalicularis of the otic capsule. The caudal free margin of the lateral occipital arch enters into the boundaries of the foramen magnum (figs. 1, 2, 3). Its ventral portion forms the larger part of the occipital condyle; its dorsal portion, a rough angular process directed medially. The processes of opposite sides mark the dorsal limit of the foramen magnum at this stage and afford attachment to the spino-occipital membrane. Dorsad of the level of these processes the lateral occipital arches are continued into the broad, curved posterior portions of the parietal plates, between which the tectum posterius extends in an arch from side to side.

Within the cranium, at the junction of basal plate and lateral occipital arch, is a rounded prominence, the cartilaginous precursor of the tuberculum jugulare, separating the entrance to the hypoglossal canal from the fossa occipito-canalicularis (fig. 1). At its cephalic end, this elevation, broadening considerably, becomes continuous with the basi-vestibular commissure (figs. 9, 10, 19). This commissure is united with the otic capsule at the boundary between the medial wall of the prominentia utriculo-ampullaris inferior and the cochlear capsule, and forms here a prominence making the posterior wall of the internal acoustic meatus. The jugular tubercle, which presents much more the form of a ridge than of a tubercle, stands within the cranium opposite the paracondyloid process. Both processes contribute to the formation of the deep caudal wall of the jugular foramen, a relation observed by von Noorden in the human embryo. The occipito-canalicular fossa lies between the inferior ampullary eminence and the basivestibular commissure and connects the sigmoid sulcus with the jugular foramen.

The jugular foramen (figs. 1, 2, 12) when viewed from within the cranium is crescentic, the convex side being formed caudally by the tuberculum jugulare and medially by the commissura basivestibularis; the concave margin, directed laterad, is constituted by the medial wall of the prominentia utriculo-ampullaris inferior. The vena jugularis interna and the accessorius, vagus

and glossopharyngeus nerves pass straight through the foramen. Although the paracondyloid process forms a deep wall for the foramen posteriorly, there is no inclination forward of this process nor a lamina alaris with attendant horizontal course of the nerves. On the extracranial surface, the foramen is divided into cephalic and caudal parts by an angular process of the cochlear wall (figs. 12, 19). The compartment posterior to the process is occupied by the jugular vein and the group of nerves; the anterior compartment, filled with connective tissue, lies within the fenestra perilymphaticum and forms a communication between the cavum cochleae and fossa occipito-canalicularis; it is the beginning of the aquaeductus cochleae.

The hypoglossal canal (figs. 1, 2, 12) transmits the three previously united ventral roots of the hypoglossal nerve. It runs in a ventro-lateral direction, beginning caudad and medialward of the jugular foramen and terminating on the external surface at the level of the medial edge of the paracondyloid process.

The broad, square paracondyloid process (figs. 2, 3, 12, 19) projects widely from the ventral part of the pars lateralis of the occipital region, with ventral and lateral free edges, cephalic and caudal surfaces. The narrow cephalic surface enters into the wall of the jugular foramen; the ventral broad free edge is continuous medially with that part of the extracranial surface lying between the jugular foramen and the hypoglossal canal.

The foramen magnum (figs. 1, 2) is hexagonal with rounded angles, the sides consisting of the free caudal margins of the basal plate and lateral occipital arches and, dorsally, the edge of the spino-occipital membrane. The foramen is divisible into a large ventral part included between the condylar portions of the occipital arches, and a smaller dorsal region extending thence to the spino-occipital membrane. The lateral boundaries of these two divisions come together in a notch of the lateral occipital wall just dorsad of each condyle (figs. 1, 2, 3, 4). A number of small veins in the connective tissue of these notches, were connected with the sinus transversus. The plane of the whole foramen magnum is nearly transverse to the longitudinal axis of the posterior half of the cranium; however,



the positions of the planes of the dorsal and ventral divisions differ from one another, that of the ventral, condylar part is transverse, whereas that of the dorsal division is very oblique. The latter faces ventro-caudad and forms an angle, open caudally, of approximately 145 degrees with the plane of the ventral part of the foramen (fig. 3).

The occipital condyles (figs. 2, 3, 19) are a pair of oval eminences only slightly raised above the level of the ventro-lateral margins of the foramen magnum. Each condyle extends at first laterally from the incisura occipitalis anterior along the caudal margin of the basal plate to the level of the hypoglossal canal, then dorso-laterally upon the lateral occipital arch. The basilar portion is broader but shorter than the lateral which stretches out upon the occipital arch as far as the deep notch mentioned above (p. 291). The lateral and basal parts of the condyle are connected with the lateral mass and ventral arch of the atlas by intervening mesenchyma; an articular cavity is not present.

The tectum posterius (figs. 1 and 2) is a slender transverse bridge of cartilage arching over the brain and connecting the parietal plates from side to side. It is far from the auditory capsules caudally and dorsally, standing closer to the occipital side walls. The tectum expands laterally in joining the parietal plates, becomes narrow in its middle opposite the medulla oblongata. The caudo-ventral margin of the tectum is concave from side to side, bounds the incisura occipitalis posterior and affords attachment to the spino-occipital membrane; the cephalic, and at the same time dorsal, margin is straight, presenting no indication of a processus ascendens.

The term incisura occipitalis posterior (fig. 1) is given tentatively to the bay extending from the foramen magnum to the tectum posterius. Its lateral boundaries are the caudal margins of a pair of cartilaginous plates continuous with the occipital walls ventrally, and with the laminae parietales anteriorly. This notch is filled by the spino-occipital membrane.

*Hypoglossal nerve.* Three ventral roots unite to form a single nerve in the embryo of 23.1 mm. A dorsal root opposite to,



but not united with, the third ventral root, arises from the spinal cord between the atlas and occipital arch. In smaller specimens further evidences of a dorsal root and ganglion were observed. This component was best developed in an embryo of 15 mm. (W. U. C. ser. 52, sl. 22-24) in which the dorsal root joined the third ventral root before reaching the hypoglossal foramen.

*Atlas and epistropheus*

In connection with the occipital region some observations on the epistropheus and atlas should be mentioned. These vertebrae have not reached their full chondrogenous development, the neural arches specially being very incomplete (fig. 9). The dens epistrophei is relatively very much longer in the embryo of the present stage than in the adult. Its relations are also different in that it projects beyond the anterior arch of the atlas so far cranial as to enter a little way into the cranial cavity through the occipital foramen (figs. 9, 14, 19). The atlas is remarkably massive. Just cephalad of the neural arches is a pair of processes of special interest. These project dorsad from the spot where the neural and anterior arches meet (the future lateral mass), inclining a little toward the median plane, and end in blunt extremities. These atlantal processes, which are the cartilaginous precursors of the little bridges of bone of the adult atlas, completing the circumferences of the foramina atlantalia, form, with the neural arches, a notch on each side lodging the ganglion of the first spinal nerve and the vertebral artery. The articular surfaces of the atlas for the occipital condyles are formed at the meeting place of the neural and anterior arches. Two parts enter into the formation of the articular surfaces: (1) a thickening at the junction of the neural and anterior arches, which meets the basal portion of the occipital condyle; (2) the atlantal process which articulates with the lateral part of the condyle.

*Basal plate and occipital region in smaller embryos*

In van Wijhe preparations of embryos of 10 mm. (fig. 5) there appears in the floor of the occipital region a pair of faintly stained, elongate parachordal plates. These are united anteriorly by a commissure beneath the notochord, but are separated across the midline in the rest of their extent. Each plate is thickened at its caudal end, and is then continued laterally into the occipital arch. The lateral margin of the parachordal plate presents a prominent angle subdividing it into an anterior oblique part opposite the mesenchymal cochlear capsule, and a posterior straight part opposite the jugular vein. In the angle between the straight part of the margin and the lateral occipital arch are the three roots of the hypoglossal nerve. Between the parachordal plates and behind their commissure is a vacuity traversed by the notochord. This space is constricted at its middle by opposite projections from the medial edges of the parachordals. The notochord extends forward as far as the hypophysis and appears to lie in a tract of blue-stained tissue which terminates just behind the hypophyseal cartilage. Each lateral occipital arch is slender and cylindrical medially where it joins the parachordal plate, expanded and flat laterally at its free extremity. Where these two parts come together the cartilage is only slightly stained. The lateral arches at this stage lie far removed in a caudal direction from the otic capsules which are represented by parts of the anterior and posterior semicircular canals.

The atlas has the form of a transverse arch open dorsally. Each lateral extremity of the arch, deeply stained, is in the form of a broad plate presenting two processes, the neural and atlantal. The middle, slender hypochordal part of the arch is only slightly stained. The epistropheus likewise forms an arch, the ventral medial part of which includes the notochord. Here a darkly stained tract on each side is separated from the notochord medially and the deeply stained neural arch laterally by very lightly stained zones. The two tracts near the notochord are the beginnings of the centrum of the epistropheus. Just cephalad of the epistropheal centrum and separated from

it by a transverse plane of unstained tissue is the broad conical mass of young cartilage surrounding the notochord, the beginning of the atlantal centrum. Between the apex of the latter and the commissure of the parachordals the notochord stands free of any chondrified tissue as evidenced by the absence of the blue stain about it.

Transverse sections of an embryo of 10.6 mm. (fig. 15) make clear the relations of the notochord, dens epistrophei and parachordal plates at the stage of cartilaginous structure of the latter. The parachordals are connected across the median plane, ventrad of the notochord by loose tissue. This tissue presents more and more the characters of mesenchyma when traced toward the median plane, and more and more the condition of cartilage when followed toward the parachordals into which it passes. An imperfect basal plate is thus formed in the occipital region which extends from side to side ventrad of the notochord and which passes laterally into the lateral occipital arches. Figure 15 shows the notochord covered dorsally and laterally by a layer of cellular tissue lying upon the dorsal surface of the stretch of mesenchyma connecting the parachordal plates. When followed caudad this layer increases in thickness, especially on the dorsal side of the notochord, and passes over into the mesenchyma of the centrum of the atlas.

In van Wijhe preparations of 12 mm. (fig. 6) the most important differences from the preceding stage in the posterior part of the cranium are the presence of a hypoglossal foramen, the beginning of the parietal plate and the slender cartilaginous basal plate of the otic region. The hypoglossal foramen has resulted from the development of a bar of cartilage uniting the lateral angle of the parachordal plate and lateral occipital arch outside the roots of the twelfth nerve. The beginning of the parietal plate appears standing free, dorsad of the posterior semicircular canal of the otic capsule. The basal plate of the otic region is continuous behind with the primary commissure of the parachordals, terminates in front in an expansion which extends nearly as far as the cochlear wall, and anteriorly projects somewhat dorsad of the hypophyseal cartilage.

In sections of 12 mm. embryos (fig. 17) the notochord, as it enters upon its cranial course, lies at first dorsad of the still imperfect basal plate, then sinks into the otic portion of the plate; it is now surrounded on all sides by cartilage at the level of the primary parachordal commissure. The notochord emerges from the basal plate at its free anterior end; which is inclined dorso-cephalad, and terminates in the mass of mesenchyma which fills the interval (*fenestra basiscranialis posterior*) between the expanded end of the basal plate and the hypophyseal cartilage. Where the notochord enters the cranium it is surrounded, as in the preceding stage, by a layer of mesenchyma which extends caudally to join with the chondrifying tissue forming the centrum of the atlas. The centrum stands in the same transverse plane as the arches of the atlas, from which it is separated by a stratum of less compact mesenchyma. Sagittal sections show a plane of densely packed nuclei separating the centrum of the atlas from the centrum of the epistropheus already laid down in young cartilage. Chondrification of the hypochordal arch of the atlas is less advanced than that of the tissue beneath the notochord between the caudal margins of the parachordal plates; in the latter young cartilage is present, while in the hypochordal arch of the atlas mesenchyma alone is to be found.

Between the caudal margin of the parachordal plates and the lateral mass of the atlas is a transverse stretch of deeply staining tissue, dense laterally and thin medially where it meets the mesenchyma about the notochord. Its later history is not clear.

Two marked advances in development of the floor of the cranium appear in a van Wijhe preparation of 15 mm. (fig. 7). First, the basal plate is nearly perfected, the unchondrified part in the occipital region being now considerably reduced. The bar completing the boundaries of the hypoglossal foramen stands opposite the primary commissure of the parachordals and is thickened at its origin, just where the jugular tubercle and paracondyloid processes later are developed. At the same spot a slight projection forward toward the cochlear capsules, medial to the vena jugularis, is probably the beginning of the basi-

vestibular commissure. Second, the anterior expanded extremity of the basal plate is united with the hypophyseal cartilage forming the crista transversa. The carotid artery as it enters the cranium is pressed against the side of the expanded end.

The dorsal extremity of the lateral occipital arch is not united anteriorly with the pars canicularis of the otic capsule; it is, however, joined with the parietal plate which now presents a prominent dorso-medial angle, the beginning of the tectum posterius.

In the atlas the hypochordal arch is partly chondrified. It is composed of two bars, right and left, which are continuous at each side with the lateral masses and approach each other medially. The lateral mass has been formed from the expanded end of the hypochordal arch of the preceding stage. The centrum of the atlas has united with that of the epistropheus so that a dens epistrophei can be described. The anterior extremity of the latter projects cephalad beyond the transverse plane of the atlantal hypochordal arch. The lateral arches of the epistropheus are connected with the centrum by a lightly stained zone.

Sagittal sections of the embryos of 15 mm. show that the basal plate is chondrified and is continuous with the floor of the cranium in the hypophyseal region (fig. 18). Here, the anterior end of the plate is still recognizable as an elevation, the crista transversa, behind the hypophysis. The bent extremity of the notochord lies upon the transverse crest imbedded in a hill of mesenchyma. In the caudal region of the basal plate the notochord still occupies a position near the intracranial surface, covered by connective tissue continuous with the dens epistrophei. A superficial constriction marks the original plane of fusion of the centra of the first and second vertebrae. Chondrification of the dens is less advanced toward its extremity than at its base. Since the last stage described chondrification has extended forward in the mesenchyma about the notochord, in consequence of which the extremity of the dens has been lengthened and now lies upon the caudal margin of the basal plate.



The hypochondral arch of the atlas is composed of young cartilage, least developed toward the midplane and continuous, right and left, with the lateral masses.

In embryos of 17 to 20 mm. (fig. 8) the basal plate is chondrified from side to side. Boundaries of the jugular foramina are established by the presence of the basicochlear commissures and the more complete union of the lateral occipital arches and the otic capsules. In relation to the hypoglossal foramen, the jugular tubercle appears anterior, the paracondyloid process lateral; the one within the cranium, the other outside. The basal foramina are all relatively large. A long, wide basi-cochlear fissure is limited anteriorly by the basi-cochlear commissure which separates it from the carotid foramen. The tectum posterius is represented in cartilage by a spur springing on either side from the parietal plate. The hypochondral arch of the atlas is complete from side to side. The dens epistrophei is further chondrified cranially.

*Otic region. Embryo of 23.1 mm.*

The otic capsule, externally, presents two well defined subdivisions, one serving as the framework of the semicircular canals, the other enclosing the cochlear canal (figs. 1, 2, 3). The vestibule, on account of its intimate relation both with the pars canalicularis and the pars cochlearis, will be included in the description of these subdivisions.

Regarding the position of the otic capsule at the stage represented by the model, a departure from the mammalian type is to be noted. The long axis of the whole auditory capsule of mammals is, as Gaupp has indicated, directed from behind and laterally, forward and medially; just the opposite of what occurs in lower vertebrates. The long axis of the otic capsule of the cat embryo of 23.1 mm. is inclined somewhat transversely (figs. 1, 2). It is true that the broader canalicular part reaches caudally a little way beyond the cochlear division, but in the cephalic direction both attain almost to the same level.

Regarding the relation of the otic capsule to the walls of the cranium, the pars cochlearis lies wholly in the floor and the pars

canicularis stands nearly upright in the lateral wall. The external surface of this part rises straight toward the lamina parietalis; the internal surface inclines from above, ventrad and a little medialward. The cochlear capsule, very large and rounded, lies beneath the brain in the base of the skull, approaching closely its fellow opposite, the two making up almost the entire cranial floor in the otic region.

At its circumference the otic capsule is connected with neighboring parts of the side wall and base of the cranium by broader or narrower stretches of cartilage with intervening vacuities. The several connections between the otic capsule and the occipital wall and basal plate as well as the related openings have already been described (p. 289): There remain to be noted the bonds between the capsule and parietal plate, and certain parts in the orbito-temporal region.

The dorsal, narrow margin of the pars canicularis is, in large part, free, forming the ventral boundary of the foramen jugulare spurium (figs. 3 and 4). Cephalad and caudad of this narrow fissure it is connected with the lamina parietalis; in the former situation, by the commissura parieto-capsularis, in the latter place with that part of the parietal plate which is continuous ventrally with the occipital wall. Between the cephalic pole of the pars cochlearis and the ala temporalis is the commissura alicochlearis which limits the carotid foramen laterally. Between this opening and the fissura basicochlearis posterior the cochlear capsule is united continuously with the basal plate (p. 288).

#### *The pars canicularis. Outer form*

Oval and laterally compressed, the massive pars canicularis stands in the side of the skull, contributing a large part of its lateral wall. It presents an extracranial lateral surface, anterior and posterior surfaces mainly within the cranium, and a narrow arched dorsal margin. A vestibular region unites this subdivision of the otic capsule with the pars cochlearis.

The lateral surface (figs. 3, 4) convex and smooth, shows only slight elevations corresponding to the semicircular canals.

Rounded prominences at the cephalic and caudal poles, which appear on both the medial and lateral aspects of the otic capsule, are identified as the *prominentia utriculo-ampullaris superior* and the *prominentia utriculo-ampullaris inferior*, respectively (fig. 10). The lateral surface of the *pars canalicularis* terminates ventrally in a broad irregular ridge, the *crista parotica*, which, projecting basally, extends from the superior utricular prominence the full length of this surface. In its caudal half the *crista parotica* (figs. 2, 20) gives rise to an oval elevation connected medially with the base of Reichert's cartilage. The elevation bears a close relation to the future *processus mastoideus*, which in the cat belongs exclusively to the otic region; but that it gives rise to it I am unable to say. The parotic crest, especially in its caudal two thirds, projects from that part of the ear capsule lodging the lateral semicircular canal. In its cephalic part is a slight depression of its lateral surface, the *fossa incudis* (fig. 3) where the cartilaginous incus is connected by a ligament. A *tegmen tympani* at the stage represented by the model is not present. Further relations of the *crista parotica* with Reichert's cartilage, and the facial canal will be discussed under these subjects. A deep groove separates the *crista parotica* from the *pars cochlearis*. This can be traced anteriorly beyond the limits of the crest upon the inferior cochlear prominence toward the *foramen faciale*; posteriorly as far as the jugular foramen. The facial foramen (fig. 1) transmitting the N. *facialis*, is directed antero-laterally and lies without the plane of the *membrana limitans* (vide infra). Here is formed the geniculate ganglion and the origin of the great superficial petrosal nerve. In its anterior part the groove lodges the facial nerve and is identified as the *sulcus facialis*. At the base of Reichert's cartilage it becomes narrow through the approximation of this cartilage toward the *crista parotica* and the wall of the cochlea, here forming the promontory (fig. 12). Since, at this spot the nerve makes its exit from the groove, it is to be regarded as the *foramen stylomastoideum primitivum*. Caudad of this foramen, the groove is broad and deep, filled with connective tissue and occupied to a small extent by the hinder part of the

cavum tympani. In it lies also the vena capitis lateralis. In the model the expanded posterior part of the groove communicates freely with the great foramen cochleae, opening at its medial side.

The intracranial surface of the pars canicularis is depressed below the general surface of the cranium and presents an uneven contour (fig. 1). Ventrally it passes into the pars cochlearis. At the place of transition a great quadrilateral fenestra acustica transmits the divisions of the N. acusticus. A ridge, eminentia cruris communis, produced by the crus commune of the anterior and posterior semicircular canals, subdivides the intracranial surface into anterior and posterior faces. The eminence presents the foramen endolymphaticum and inferiorly passes into the prominentia utricularis. The latter, corresponding to the utricle and saccule, reaches below to the acoustic window. The posterior surface of the pars canicularis looks caudad, mesad and slightly ventrad, contrasting in its position therefore with the caudally and ventrally directed corresponding surface in mammals generally. It passes caudally into the medial surface of the lateral occipital arch and ventrally into the prominentia utriculo-ampullaris inferior. The posterior surface is hollowed to form the sigmoid sulcus, occupied by the sinus transversus. The anterior surface of the pars canicularis presents the fossa subarcuata anterior limited anteriorly and dorsally by the prominentia semicircularis anterior. This prominence begins at the dorsal pole of the pars canicularis and curves anteriorly and ventrally to the prominentia ampullaris superior. On this surface, also, the stout commissura suprafacialis<sup>2</sup> is found, connecting the medial wall of the prominentia utriculo-ampullaris superior with the anterior part of the roof of the pars cochlearis, and participating in the ventro-caudal circumference of the spheno-parietal fenestra. It is continued dorsally into a

<sup>2</sup> In van Wijhe preparations of embryos 17 to 30 mm. long, the suprafacial commissure appears deeply stained and separated from the pars canicularis by a zone of lightly stained cartilage. It continues directly into the commissura orbito-parietalis where this is united with the otic capsule (commissura parieto-capsularis) (fig. 9).

prominent ridge which joins the orbito-parietal commissure (figs. 1, 3). The suprafacial commissure constitutes the roof of the short first portion of the facial canal, the floor of which is made by a cartilaginous septum between it and the cavum cochleae. The canal leads from the meatus acusticus internus to the foramen faciale. The dorsal circumference of the pars canalicularis is partly free, partly continuous with the cranial wall. Posteriorly it passes into the occipital wall; anteriorly it is connected with the commissura parieto-capsularis. Between these connections the dorsal margin is free, forming the ventral boundary of the foramen jugulare spurium. Upon the medial side of the dorsal circumference is a groove occupied by the transverse sinus and its main anterior tributary.

#### *Pars cochlearis*

The most conspicuous object of the cranial floor as presented by the model is the huge, rounded bulging of the pars cochlearis of the auditory capsule (figs. 1, 2). The strong walls of this shell enclose the cochlear canal and the saccule. The form of the cochlear capsule is not quite round, but longer in a direction from behind, forward and medialward. Upon the ventral, extracranial surface a shallow groove, sulcus septalis, extends from behind and laterally, medially and anteriorly. Upon the caudal aspect is the large foramen cochleae. Where the cochlear and canalicular parts come together are three openings: the fenestra vestibuli, appearing outside the cranium, the fenestra acustica, within the cranium and the foramen perilymphaticum, appearing partly inside and partly outside the skull. The pars cochlearis shows no sharp boundary toward the pars canalicularis; as stated above, the two are continuous in the region of the vestibule. On the surface, this common vestibular zone corresponds to the intermediate stretch which includes the sulcus facialis laterally, and the region of the fenestra acustica, medially. The connections between the cochlear capsule and the floor of the cranium have already been described (p. 288).

*Foramina acustica* (figs. 10, 11). A large quadrilateral opening, fenestra acustica, the future porus acusticus internus,



is situated upon the medial aspect of the ear capsule, partly in the wall of the cochlea, partly in that of the vestibule. Its plane faces dorsad and mesad, the lateral margin made by the *prominentia utricularis* being considerably higher than the medial cochlear edge. The fenestra leads into a short, imperfectly formed *meatus acusticus internus*; within is an incomplete floor presenting two openings, one transmitting the facial nerve, the other the branches of the acoustic nerve. The former, which lies anteriorly, is the beginning of the primary facial canal. It will be recalled that this short canal passes beneath the supra-facial commissure and ends in the facial foramen outside the *chondrocranium*. The opening for the acoustic nerve is an irregular gap; for convenience of description it will be referred to as the acoustic fissure. It is shaped like a V with apex caudad, one limb lying almost wholly in the cochlear, the other in the vestibular region. The latter division, widened in the form of an oval notch, lies anterior and slightly dorsal to the cochlear division. It transmits the nerves to the utricle, and to the ampullae of the anterior and lateral semicircular canals. Its position and relations to the structures just named identify it with the superior acoustic foramen of other mammalian embryos. The larger cochlear portion of the acoustic fissure lies caudad, medialward and somewhat ventrad to the superior acoustic foramen. It gives passage to the nerve of the cochlea and the nerve to the sacculus, and is identified with the inferior acoustic foramen. In the dorsal caudal corner of the *meatus acusticus internus* is a notch opening into the cochlear part of the fissure and leading to a groove, lodging the nerve to the ampulla posterior. The notch is the forerunner of the *foramen singulare* and will be designated the *incisura singularis*.

The position and relations of the facial and acoustic foramina having been noted, it now remains to describe the walls of the internal acoustic meatus. Figures 13 and 14 show the meatus from the medial aspect. Of the stretches of cartilage intervening between the facial and acoustic foramina two are of special interest: one, constituting the primary floor of the meatus, separating the two named foramina; the other forming the incom-

plete lateral wall of the meatus. The primary floor of the meatus appears in the model as an extension from the roof of the anterior part of the cochlear cavity. It can be followed laterally from the dorsal part of the cochlear capsule at the medial margin of the fenestra acustica to pass into the first turn of the septum spirale. This part is the forerunner of the longitudinal bony ridge in the floor of the meatus of the adult, the crista transversa of human anatomy, which separates the superior cribriform area and entrance to the facial canal above from the middle cribriform area and the spiral tract of foramina below (Jayne '98, p. 200). Anteriorly it continues as the floor of the primary facial canal and at the same time the roof of the fore part of the cochlear cavity. Lateral to the facial foramen the primary floor of the meatus passes into the floor of the promontia utriculo-ampullaris superior and forms the ventral boundary of the superior acoustic foramen. The posterior free edge of the floor constitutes the cephalic margin of the inferior acoustic foramen. The lateral wall of the meatus is formed mostly of precartilage. Its free ventral margin bounds the acoustic fissure dorsally. The wall itself covers the medial aspect of the sacule. The medial edge of the meatus, derived from the roof of the cochlear capsule, stretches from the suprafacial commissure to the posterior wall and is very low. The anterior and posterior walls, on the contrary, tend to become very high toward the vestibular part of the capsule, and are formed respectively by the suprafacial commissure and an elevation made at the junction of the basivestibular commissure with the promontia ampullaris inferior and cochlear capsule.

Within the meatus acusticus internus are found, anteriorly, the nerve of the vestibule and its ganglion, together with the facial nerve, and, posteriorly, the nerve of the cochlea.

In the model of the chondrocranium, there is a small flattened surface of the cochlear capsule, lateral to the suprafacial commissure (figs. 3, 20). This, the planum supracochleare of Voit, supports the seventh nerve as it passes out of the foramen faciale, the geniculate ganglion and the beginning of the great superficial petrosal nerve. Upon it rest also the caudal part

of the semilunar ganglion and the roots of the fifth nerve. As shown by the sections, these structures are embedded in mesenchyma which extends laterally to Meckel's cartilage and medially as far as the plane of the chondrocranial wall; here, in the form of a membrane, it becomes fixed to the margins of the fenestra sphenoparietalis. For this region the name *cavum supracochleare* has been proposed by Voit. The *cavum supracochleare* and the several structures therein are excluded from the cavity of the chondrocranium by the membrane of the sphenoparietal window.

*Cavities of the otic capsule*

A frontal section through the vestibular region shows a large irregular space containing parts of the membranous labyrinth (fig. 10). This space extends anteriorly into the *pars cochlearis* as the *cavum cochleae*, lodging the cochlear duct; posteriorly into the *pars canalicularis* in the form of the *canales semicirculares*. The main room opened by the section, *vestibulum*, contains the *utricle* and *sacculle*.

*Cavum cochleae* (figs. 19, 20, 21, 22). The great oval cavity of the *pars cochlearis*, traversed by a low *septum spirale*, has already begun to assume the form of a winding canal. This may be followed from the vestibule, with which it is in wide communication, ventrally, then in a spiral course into the anterior part of the cochlear capsule. At its commencement the canal is quite wide, and its limiting cartilaginous walls incomplete through the presence of large openings; caudally, the *perilymphatic foramen*; laterally, the *fenestra vestibuli*; dorsally, the *inferior acoustic foramen*. The cochlear duct makes a little more than one complete turn and occupies but a small part of the cavity which is elsewhere filled by young connective tissue (fig. 9). This tissue extends into the *perilymphatic foramen* and to the edge of the spiral septum where, by condensation of its elements, it presents the form of a spiral membrane (fig. 22). No indication of cartilage formation was observed in this membrane in the present or in a later stage. The cartilaginous *septum spirale* takes origin in the primary floor of the internal

acoustic meatus (figs. 10, 11, 19), makes a steep descent toward the floor of the cochlear cavity, and continues as a low ridge to the extent of somewhat more than half a turn from the beginning of the septum. Its position externally, is indicated on the ventral surface of the cochlear capsule by the sulcus septalis (p. 302).

The oval fenestra vestibuli (figs. 3, 20, 21) appears externally at the bottom of a deep depression of the surface of the capsular wall, standing in a coronal plane, above and lateral to the perilymphatic foramen. Its superior and medial boundaries are very prominent; its inferior limit obscure. Within this depression is the foot-plate of the cartilaginous stapes, embedded in mesenchyma which completely fills the window. Within the cavum cochleae, the fenestra appears on an eminence of the lateral wall which projects posteriorly toward the vestibule.

The large foramen perilymphaticum (figs. 2, 12, 20) extends upon both the caudal and dorsal surfaces of the pars cochlearis and is incompletely divided by a process of the cochlear capsule into two parts, the fenestra cochleae and the aquaeductus cochleae. The former is located at the caudal pole of the cochlear prominence, stands in a transverse plane and is occupied by mesenchyma. In figure 11 it appears completely separated from the aquaeductus cochleae, but the septum between these openings is chondrified only in its medial part; laterally it is composed of precartilage. The aquaeductus cochleae, lying in a frontal plane, opens into the fossa occipito-canalicularis above (p. 291), and communicates with the cavum cochleae below. It is filled with mesenchyma, through which small veins pass to the vena jugularis interna. The aqueduct of the cochlea at the present stage is an extension of the jugular foramen forward in the form of a deep notch between the commissura basivestibularis and the prominentia utriculo-ampullaris inferior. It constitutes a vacuity in the roof of the cochlear capsule. The aquaeductus cochleae is separated from the jugular foramen proper (i.e. the posterior part transmitting the vein and nerves) by the free angular projection of the cochlear wall above mentioned (indicated by \* in figs. 2, 12, 19).



*Vestibulum.* A frontal section through the middle of the vestibular region reveals a space within the cartilage stretching antero-posteriorly and lying nearer the medial than the lateral margin of the section (fig. 10). This space, a part of the future vestibule, is narrowed in its middle by the encroachment of a massive projection of the lateral wall and is expanded rather widely in front and behind this. The anterior wide room, containing in the section the utricle and recessus utriculi and the ampullae of the anterior and lateral semicircular ducts, will be described under the name of the *cavum vestibulare anterius*. The posterior room includes, in the section, the posterior part of the utricle, the ventral part of the ductus endolymphaticus, the beginning of the canalis utriculi-saccularis, the sinus inferior, ampulla posterior and the crus simplex of the ductus semicircularis lateralis. This room will be further considered under the name of the *cavum vestibulare posterius*. The parts of the membranous labyrinth mentioned do not fill completely the space limited by the cartilaginous walls of the *pars vestibularis*, but leave an interval filled by mesenchyma representing the future perilymphatic space.

*Cavum vestibulare anterius* (figs. 10, 11). This large, irregularly shaped cavity occupies the dorsal part of the vestibular region. Its contents have been noted. Its anterior wall swells out as the *prominentia utriculo-ampullaris superior*. This corresponds to an anterior extension of the cavity called *recessus ampullaris anterior* containing the ampulla anterior. The floor is broad, slopes ventrad in a medial direction, then drops abruptly to continue into the lateral cochlear wall. At this spot is located the *fenestra vestibuli* (figs. 19, 20). The medial wall bulges slightly into the cranial cavity forming the *prominentia utricularis*, in conformity with the subjacent surface of the utricle and saccule. The ventral free edge of this wall forms the dorsal boundary of the superior acoustic foramen as already described (p. 304). The lateral wall presents a dorso-ventral ridge which projects into the cavity, fitting into the angle between the ampulla lateralis and ampulla anterior. The considerable space behind the ridge, containing the greater part of the lateral am-



pulla opens posteriorly into the canalis semicircularis lateralis. The posterior wall is formed by the mass of cartilage already referred to which projects medialward from the lateral wall of the otic capsule (fig. 11). This crista intervestibularis, as it may be termed, intervenes between the anterior and posterior vestibular caves, but fails to meet the medial vestibular wall; there is left a communicating space between the two divisions occupied by a part of the utricle. Ventrally, the crista intervestibularis enters into the roof of the lateral semicircular canal; dorsally it becomes continuous with the massa angularis, the great body of cartilage encompassed by the semicircular canals. The dorsal wall of the cavum vestibulare anterius is made by that portion of the angular mass which fills the concavity of the canalis semicircularis anterior.

*Cavum vestibulare posterius.* In form, the posterior division of the vestibule is oval dorsally, narrow and tunnel-shaped ventrally, expanding into a roomy space in the region of the fenestra cochleae. The posterior wall bulges in adaptation to the adjacent recessus ampullaris posterior, forming on the surface of the ear capsule the prominentia utriculo-ampullaris inferior. The ventral part of the posterior wall presents the posterior orifice of the lateral semicircular canal. The dorsal part, at its junction with the roof of the cavum vestibuli posterius, shows the orifice of the posterior semicircular canal. The medial wall, continuous with the medial wall of the anterior cave, corresponds to that part of the otic capsule marked on the surface by the beginning of the prominentia cruris communis and the incisura singularis. It presents a ventral free edge looking into the inferior acoustic foramen. The dorsal wall consists of that part of the massa angularis intervening between the canalis semicircularis posterior and the vestibule. In the highest part of the roof are two openings:—the great circular orifice of the cavum cruris communis, containing the crus commune of the anterior and posterior canals; the small irregular hole, foramen endolymphaticum, looking medialwards and transmitting the ductus endolymphaticus. A floor is present in the caudal half only, beneath the ampullary end of the posterior semicircular duct;

where the floor is wanting anteriorly there is a wide communication with the *cavum cochleae*.

The semicircular canals (figs. 2, 9, 10, 11, 19) are cylindrical tunnels of a diameter from two to two and one-half times that of the contained membranous ducts. The future perilymphatic space between the ducts and walls of the canals, is filled with mesenchyma. Within the compass of the canals is the body of cartilage to which Gaupp has given the name *massa angularis*. The lateral semicircular canal lies in a frontal plane; its floor, lateral and medial walls go into the cartilage of the *crista parotica*; its roof is formed by the *crista intervestibularis*. The plane of the anterior semicircular canal is dorso-ventrad inclining lateromesad from before backward. Within its concavity is that part of the *massa angularis* which forms the roof of the *cavum vestibulare anterius* and which corresponds to the *fossa subarcuata* of the intracranial surface of the *pars canalicularis*. The posterior semicircular canal also runs in a dorso-ventral plane, which, however, is inclined from behind and laterally, forward and medially. It includes within its concavity that part of the *massa angularis* forming the roof of the *cavum vestibulare posterius* and which corresponds on the intracranial surface of the *pars canalicularis* to a *fossa*. From the bottom of this *fossa*, which is situated behind the *prominentia cruris communis*, a canal, transmitting some blood vessels, leads to a great vacuity within the angular mass.

The *crista intervestibularis* (fig. 11) is a projection of the base of the *massa angularis*. It begins just dorsad of the *fenestra vestibuli* as a ridge lying between the *ampulla lateralis* and the *sacculi*; it reaches its maximum height opposite the *ductus utriculo-saccularis*.

The *massa angularis* (figs. 2, 10, 11), filling the space between the three semicircular canals and the vestibule, is composed of hyaline cartilage, fully formed. Its relation to the walls of the canals and vestibule have already been described. Ventrally the mass becomes continuous with the *crista intervestibularis*. The angular mass is not continuous throughout, being broken in its central portion by a space occupied by connective tissue

and blood vessels (indicated by \*\* fig. 2). The latter find a passage to the cranial cavity by the opening in the fossa behind the eminentia cruris communis (vide supra); veins passing out of this opening join the sigmoid sinus. The space extends cephalocaudad and dorsad; approaching the anterior and posterior semicircular canals and the crus commune, and reaching ventrad as far as the level of the roof of the vestibular cavities and the crista intervestibularis. It does not at any point communicate with the vestibule.

*Lamina parietalis* (figs. 1, 2, 3, 4). This plate of cartilage stands in a sagittal plane dorsad of the pars canalicularis. The concave extracranial surface gives origin to part of the temporal muscle; the convex intracranial surface is smooth. The parietal plate is broad anteriorly and posteriorly and narrow between where it lies dorsad of the pars canalicularis of the otic capsule. It goes over anteriorly into the commissura orbito-parietalis posteriorly into the lateral occipital wall, and, in connection with these two parts, is fixed to the pars canalicularis. Between the four elements named is the foramen jugulare spurium, narrow and curved around the dorsal circumference of the otic capsule. Some minute veins traversing the foramen connect the transverse sinus with extracranial veins. The foramen of the right side is subdivided by a cartilaginous connection of the parietal plate and pars canalicularis. The hinder of the two resulting foramina is located opposite the summit of the posterior semicircular canal. In van Wijhe preparations of embryos 24 to 30 mm. in length, union of the parietal plate and pars canalicularis was observed dividing the spurium jugular foramen into two parts. This connection was found on one side or the other, rarely on both, occurring, as in the stage modeled, at the summit of the anterior semicircular canal. The parietal plate at this level is very narrow, is notched in its dorsal margin and, compared with the regions anterior and posterior, is less chondrified.

*Otic region in smaller embryos*

In cat embryos of 17 mm. (H. E. C. ser. 492 and van Wijhe preparations, fig. 8) both pars canalicularis and pars cochlearis are largely chondrified and stand in connection with each other. At their margins they are united with the floor and walls of the cranium by commissures between which openings are found. The basal plate, sella turcica, parietal plate, orbito-parietal commissure and occipital wall, parts adjacent to the auditory capsule, are all formed of cartilage. The cartilaginous structure of the ventral portion of the occipital wall and that of the floor of the cranium are further advanced in development than the cartilage in the remaining parts.

In the pars canalicularis, cartilage has formed upon its lateral surface, anterior, posterior and dorsal margins, around and between the semicircular ducts; its medial surface is chondrified about the circumference and crus commune. The prominentiae utriculo-ampullaris superior and inferior present cartilaginous walls, that of the last named being continuous ventrally with the cochlear wall. A chondrified medial wall of the vestibule forms the dorsal boundary of the future internal acoustic meatus. At the margins of the pars canalicularis the sections show that planes of prochondral tissue separate it from the parieto-capsular commissure in front and lateral occipital arch posteriorly.

As shown by the sections through the cochlear capsule, pre-cartilage is everywhere present excepting at the anterior and posterior poles. At these two spots the cochlea is united with the cartilage of the cranial base by commissures, but between the commissures it is separated from the basal cartilage by an extensive basicochlear fissure filled with mesenchyma. The roof of the cochlear capsule is considerably further developed than the floor and is nearer the cartilaginous state toward the pars canalicularis than in the direction of the median plane. At the posterior pole young cartilage can be followed from the prominentia utriculo-ampullaris inferior medially and caudally into the cochlea. This area lies between the fenestra acustica and the foramen perilymphaticum; from it the basivestibular commis-

sure, just caudad, can be differentiated by its more advanced state of development. At the anterior pole, cartilage formation is found in connection with the suprafacial commissure. Where this bar joins the cochlear capsule two regions of the capsule, distinguished by their different states of development, can be seen in the sections. One region, that next to the epithelial cochlear duct is composed of mesenchyma which passes peripherally into precartilag. The other is a circumscribed area superimposed upon the part just mentioned. Its form is somewhat circular in sagittal sections; it is composed of young cartilage. This area occupies the most cephalic part of the cochlear capsule. It can be followed medially through sections 198-192 (H. E. C. ser. 492) of the anterior portion of the pars cochlearis as a more or less distinct tract; further on it becomes completely fused with the deeper stratum of the wall of the *cavum cochleae*. In sections 191-186 the anterior pole of the cochlear capsule appears as a simple curved plate of young cartilage. In section 185 this is joined with the alicochlear commissure. The relations of the cochlear capsule to the latter and to the suprafacial commissure will be understood by reference to figure 8. When traced in the lateral direction, the curved plate in question goes over into the suprafacial commissure, with which it conforms in the degree of cartilaginous differentiation, outward size and shape. A second connection between the anterior pole of the cochlear capsule and basal cartilage is presented at this stage by the cartilaginous basicochlear commissure, or *synchondrosis*, which forms the posterior boundary of the carotid foramen. The parietal plate is partly separated from the canalicular division by the foramen *jugulare spurium*. It is continuous through young cartilage or precartilag with the occipital arch and *pars canalicularis* posteriorly and with the *commissura orbito-parietalis* anteriorly. The *tectum posterius* is represented by a short process of the *lamina parietalis*.

Cartilage is found in the otic capsules of a cat embryo of 15 mm. (fig. 7) about the walls of the semicircular ducts and the vestibule, as indicated by the deep blue stain in van Wijhe preparations; the cochlear capsule is unstained. The basivestibular



commissure, the alicochlear commissure and the basicochlear commissure behind the carotid artery are likewise unstained; the commissura orbito-parietalis is represented by a faintly stained strip of cartilage independent of both the orbital plate and otic capsule. The parietal plate is a triangular cartilage surmounting the extremity of the occipital arch and joined to it; it is unconnected with, though close to, the pars canicularis. Its medial and dorsal angle is the beginning of the tectum posterius. Sections (H. E. C. ser. 400) show the material of the basal plate and floor of the sella turcica to be young cartilage. The basivestibular, alicochlear and basicochlear commissures are composed of mesenchyma. The suprafacial commissure appears as a bar extending from the anterior pole of the pars canicularis over the nervus facialis to the anterior part of the cochlear capsule. It is composed of precartilage, easily distinguished from that of the otic capsule by the more abundant, clear ground substance between the nuclei. The relations of this commissure are of high interest. Followed laterally across the facial nerve it meets the pars canicularis over the prominentia utriculo-ampullaris superior. It then passes without the smallest difference in degree of chondrification, without boundary of any sort, into the commissura orbito-parietalis. Chondrification of these commissures is quite uniform, and represents a state of histogenesis different from that of the otic capsule. Between the orbito-parietal commissure and the otic capsule is a plane of mesenchymal tissue. The separation of the suprafacial commissure and otic capsule is not so sharp.

When now sections are followed in series from the spot where the suprafacial commissure joins the pars cochlearis, toward the median plane, an equally interesting connection becomes manifest. It was shown above that in embryos of 17 mm. the cochlear end of the commissure became continuous with a stretch of precartilage in the anterior part of the cochlear capsule, corresponding in shape and state of development exactly with that of the commissure itself. In the present stage this stretch again appears and can be followed medialward and cephalad almost as far as the point of union of the commissura alicochlearis with the cochlear capsule.

Figure 6 made from a van Wijhe preparation of 12 mm. shows the otic capsule from its medial side. Only those parts appear which are chondrified. The three semicircular ducts are in large part walled by cartilage. The blue stain is most intense upon the lateral aspect, faint on the medial side of the ducts. The parietal plate is a small triangular cartilage, lying dorsad of the interval between the posterior semicircular canal and the lateral occipital arch. It is entirely independent of other cartilages of the cranium.

The first trace of chondrification of the otic capsule was found in embryos of about 10 mm. (fig. 5). In van Wijhe specimens of this stage a narrow streak of blue follows the dorsal and lateral circumferences of the anterior and posterior semicircular canals.

#### *Nerves in the otic region*

A brief description of the nerves and blood vessels which have been referred to in the description of the otic region will serve to correlate the many structures which have just been described. The roots of the trigeminal nerve pass forward over the supra-facial commissure and through the septum transversum (vide infra), to the semilunar ganglion, the caudal half of which rests upon the planum supracochleare. The abducent nerve runs along the medial side of the cochlear capsule, leaving the otic region by passing forward between this structure and the dorsum sellae. The seventh nerve, together with the vestibular and cochlear divisions of the acoustic, enter the internal acoustic meatus from before backward in the order named. The glossopharyngeal, vagus and accessory traverse the jugular foramen, behind the otic capsule.

The facial nerve, including the pars intermedia (figs. 2, 4, 20, 21), is close to the ganglion of the vestibular nerve as it lies in the internal acoustic meatus. It passes through the primary facial canal and the facial foramen to enter the cavum supracochleare and there, outside of the cavity of the chondrocranium, presents upon its dorsal side the geniculate ganglion (fig. 20). Connected with the ventral surface of the latter is the great superficial petrosal nerve. Its position is entirely extracranial.

It stretches across the lateral wall of the cochlear capsule, between the geniculate ganglion and a plexus of nerves about the internal carotid artery. In the plexus it joins the N. pterygoideus Vidiani (fig. 3). Leaving the region of the geniculate ganglion the facial nerve enters upon the second part of its cranial course. It undergoes first its characteristic bend and then passes nearly straight caudad, lying for a short distance upon the prominencia utriculoampullaris superior, then enters the sulcus facialis. Above the nerve is the lateral semicircular canal, separated by a floor of thick cartilage from the sulcus facialis. Continuing its course, backward through the groove, the nerve crosses dorsad of the incudo-stapedial articulation, separated from the vestibular window and basis stapedis which lie ventrad. Passing beneath and laterad of the origin of the stapedius muscle (originates from the roof and lateral wall of the sulcus facialis) to which it supplies a twig, the facial reaches the base of Reichert's cartilage, where, bending sharply ventrad it leaves the groove. The term foramen stylo-mastoideum primitivum has been given by Broman ('99) to the exit from the sulcus facialis bounded by the upper, proximal division of Reichert's cartilage and the ear capsule. After passing the confines of this ill defined foramen (fig. 19) the facial nerve descends upon the caudal side of Reichert's cartilage and leaves the otic region.

At the side of Reichert's cartilage the facial nerve gives off its chorda tympani branch (figs. 2, 22). This stout nerve winds about the lateral side of Reichert's cartilage, ascends somewhat and, turning mesad and cephalad, enters the region of the first pharyngeal pouch. Here it lies at first upon the medial surface of the manubrium of the cartilaginous malleus, ventrad of the insertion of the M. tensor tympani and of the incudo-stapedial articulation. Proceeding forward it passes out of the tympanic region, following closely the medial and ventral surface of Meckel's cartilage.

*Acoustic nerve.* Two chief branches were present, an anterior and posterior. The anterior ramus gives a twig to the recessus utriculi, one to the ampulla anterior, another to the ampulla externa. The posterior ramus gives rise to a ramulus sacculi, a

twig to the posterior ampulla (the two together may be regarded as a ramus medius), and continues as the ramulus basilaris. In addition to the ramulus sacculi, described by Retzius ('84), a small twig to the sacculus coming from the anterior ramus was observed.

The vagus, together with the glossopharyngeus and accessorius, form a bundle which occupies a position in the fossa occipito-canalicularis close to the lateral occipital arch and considerably posterior to the pars canalicularis of the otic capsule (figs. 1, 2, 19). The vagus lies medialward of the vena jugularis with the glossopharyngeus anterior and the accessorius posterior to it. These relations are maintained in passing through the jugular foramen, excepting that the accessorius becomes incorporated with the vagus. The jugular ganglion is found upon the roots of the vagus as it lies in the fossa occipito-canalicularis and therefore above the jugular foramen. Some distance outside the cranium the ganglion nodosum appears.

The ganglion superius of the N. glossopharyngeus (figs. 1, 2, 19 to 23) is situated partly in the recessus occipito-canalicularis, and partly upon the roots of the nerve as they lie in the cranial cavity above the recess. The ganglion petrosum, much larger, is located on the nerve below the jugular foramen and above the level of the ganglion nodosum of the vagus. The tympanic nerve arises from the upper end of the ganglion petrosum and proceeds in the direction of the foramen cochleae. This nerve runs in the thick mesenchyma between the medial epithelial wall of the first pharyngeal pouch and the foramen cochleae. After much branching, it reaches the ventrolateral surface of the posterior cochlear prominence where it enters a plexus of nerves, derived in part from the sympathetic around the internal carotid artery.

#### *Blood vessels in the otic region*

A great blood sinus runs upon the inner surface of the cranial wall in the sulcus which circumscribes the pars canalicularis. This, a forerunner of the sinus transversus system, becomes continuous at the fossa occipito-canalicularis with the vena jugularis



interna. Small tributaries enter from the connective tissue filling the foramen jugulare spurium; some of these are emissary veins. Behind the pars canalicularis the sinus is joined by a small vein formed by tributary systems, one being the plexus of veins in the mesenchyma which fills the vacuity of the massa angularis (p. 309). A plexus of veins in the facial sulcus empties into a large vein which, running backward in company with the facial nerve, joins the internal jugular. An extensive network of veins within the mesenchyma of the cavum cochleae is drained in part by two veins which pass to the vena jugularis interna by way of the aquaeductus cochleae.

Vestiges of a stapedia artery are present in the embryo of 23.1 mm., perforating the stapes (fig. 21). These can be followed anteriorly toward the terminal branch of the internal maxillary artery and posteriorly toward a plexus of small vessels on the ventral surface of the cochlear capsule, which is connected with an offset from the internal carotid artery. In an embryo of 15 mm. the stapedia artery is a relatively large branch of the A. carotis interna, extending forward through the mesenchymal beginning of the stapes to the semilunar ganglion whence it continues as the mandibular artery.

The internal acoustic artery arises from the basilar and enters the internal acoustic foramen together with the acoustic and seventh nerves, giving off several branches to the membranous labyrinth.

*Orbito-temporal region. Embryo of 23.1 mm.*

The basis cranii (figs. 1, 2, 3) of the orbito-temporal region is an unbroken bar of cartilage continuous with the basal plate of the otic region posteriorly, and with the nasal septum of the ethmoidal skeleton anteriorly. The extracranial surface, strongly convex from side to side looks toward the pharynx, from which it is separated by an interval filled by wide-meshed mesenchyma. The intracranial surface presents in its posterior one-third the sella turcica with the root of the ala temporalis on each side, and in front of this, in successively higher planes, the two roots of the ala orbitalis, the optic foramen intervening. At the level of the



sella turcica the basis cranii is bent in a sagittal plane so that the otic and orbital portions form an angle, open ventrally. Also, where the orbito-temporal portion of the base goes over into the septum nasi, a similar angle is formed.

At the level of the sella turcica the base is flattened dorso-ventrally, becomes thicker opposite the optic foramina and finally triangular in its most anterior part. Here, the two extracranial surfaces incline medially and ventrally to meet in a keel, forming a short interorbital septum which passes forward into the septum nasi.

*Sella turcica* (figs. 1, 23). The elongate, shallow pituitary fossa is limited anteriorly by a low tuberculum sellae and posteriorly by a well defined wall. The base of the latter is made by the crista transversa extending nearly to the cochlear capsules, a slight interval remaining between these parts transmitting the abducent nerve. The dorsum sellae is represented by a conical elevation of the middle of the crista transversa. Remains of a hypophyseal canal are present and are occupied by epithelial vestiges of the stalk of the anterior lobe of the pituitary body.

*Ala temporalis* (figs. 1, 2, 3, 4, 23). The ala temporalis of the 23.1 mm. embryo extends from the processus alaris of the basis cranii, opposite the sella turcica, outward beneath the semilunar ganglion; it is limited by free margins in front and behind. Its structure is of young cartilage separate from that of the processus alaris, a stratum of mesenchyma standing between them. The temporal wing consists of a larger blade-formed lateral portion, the lamina ascendens, and a small, medial pterygoid process. A separate pterygoid cartilage is present, lying mesad of the pterygoid process below the basis cranii. In connection with the description of the ala temporalis the cavum epiptericum will be considered.

*Processus alaris* (figs. 2, 23). The alar process lies in front of the carotid foramen, supports the internal carotid artery in its forward course and forms the ventral boundary of the innermost part of the spheno-orbital fissure. Laterally it extends to the temporal wing, the layer of mesenchyma above mentioned limiting the two parts (fig. 23). This layer takes an oblique direc-

tion from before backward and laterally, on account of which the ala temporalis stands in front, as well as to the side of the processus alaris. Between the latter and the anterior pole of the cochlear capsule extends a cartilaginous bridge, the commissura alicochlearis, forming the lateral boundary of the carotid foramen. It is quite distinct from the temporal wing, the two being separated by the continuation of the zone of mesenchyma just described. The processus alaris and commissura alicochlearis are, however, continuous, and together form an arch around the front and side of the carotid foramen.

The lamina ascendens (figs. 1, 2, 3, 23) of the ala temporalis is a cartilaginous plate which extends laterally and dorsally toward the commissura orbito-parietalis, but remains widely separated from it. It is somewhat triangular in form, presenting free margins, anterior and posterior, which meet laterally in a rounded free point, and a base directed toward the oblique line of junction with the alar process and alicochlear commissure. The dorsal surface slopes from an intermediate longitudinal ridge, forward and backward, thus presenting two subdivisions: an anterior face entering into the floor of the spheno-orbital fissure; and a posterior, supporting the cephalic end of the semilunar ganglion and forming the floor of the epipteric cave. The longitudinal ridge itself lies opposite the lower margin of the ala orbitalis and enters into the ventral boundary of the spheno-orbital fissure. The ventral surface of the lamina ascendens overhangs the mandible and gives origin to part of the pterygoid muscle. A short canal, the forerunner of the foramen rotundum, traverses the lamina ascendens from behind forward, giving passage to the maxillary nerve. The posterior margin of the ala is free and limits anteriorly a broad, deep fissure whose posterior boundary is the cochlear capsule. The fissure ends medially at the alicochlear commissure and, like the spheno-orbital fissure, is open laterally. To this gap, which has long been known, the name *fissura alicochlearis* may be given. Through it pass the mandibular and the great superficial petrosal nerves, the former through the incisura ovalis in the posterior margin of the lamina ascendens, the latter traversing another notch, *incisura lacera*, and

joining beneath the lamina the great, deep petrosal nerve to form the N. pterygoideus. Incisura lacera is the name proposed for the deepest part of the fissura alicochlearis, a part distinguished by certain important relations. It reaches medially toward the carotid foramen, from which it is separated by the commissura alicochlearis, and here adjoins the posterior end of the mesenchymal zone dividing the commissure from the lamina ascendens. A prominent spine of the caudal margin of the ascending plate forms its antero-lateral boundary and stands between it and the incisura ovalis. On the anterior margin of the ala temporalis, adjacent to the processus alaris, is the broad processus pterygoideus (fig. 1), in connection with which is later formed the insignificant bony lamina lateralis processus pterygoidei of the adult. The pterygoid process projects forward and toward the mid-plane, lying medial of the foramen rotundum. Its medial end is separated from the adjacent pterygoid cartilage by a layer of mesenchyma. At this spot the Vidian nerve begins to turn from beneath the lamina ascendens to gain, eventually, a position dorsal of the pterygoid cartilage and within the spheno-orbital fissure.

The base of the ala temporalis is directed obliquely from behind, forward and medialward and for the most part corresponds to the synchondrosis between the ascending plate, alar process and alicochlear commissure. Two other regions, however, must be included in the base: posteriorly, the medial free margin of the spine separating the incisura lacera from the incisura ovalis, and anteriorly, the medial free margin of the processus pterygoideus. The relations into which the base of the ala enters with the rest of the chondrocranium are, therefore, the medial corner of the spheno-orbital fissure, processus alaris, commissura alicochlearis and incisura lacera.

*Ala orbitalis* (figs. 1, 2, 3, 4). This great sickle-shaped cartilage forms, on each side, the larger part of the medial wall of the orbit and the lateral wall of the fore part of the cranial cavity. Its lateral and medial surfaces are respectively concave and convex, apparently in adaptation to the eye-ball and its adnexa. The anterior extremity is joined to the nasal capsule by the com-

missura speno-ethmoidalis; the posterior extremity is connected with the fore part of the basis cranii by two roots, named preoptic and metoptic, relative to the optic nerve which they embrace. The preoptic root is broad and straight whereas the metoptic root is narrow and bent so as to present a prominent angle of its caudal margin directed backward. The optic foramen, large and irregular oval, is bounded medially by the basis cranii, on its other sides by the orbital wing and its two roots. Through the optic foramen a short, blunt process, processus orbitalis, extends into the orbit from the cephalic edge of the metoptic root. On the right side this process lies close to the basis cranii. From the roots of the orbital wing some of the ocular muscles take origin (vide infra). The short concave, anterior margin of the wing is free and separated from the nasal capsule by the fissura orbito-ethmoidalis. This is filled by membrane and transmits the lateral branch of the nasal nerve. The extensive, convex, posterior margin, dorsally, passes into the commissura orbito-parietalis connecting the orbital wing with the parietal plate, and ventrally is free in the anterior boundary of the fenestra speno-parietalis.

The speno-orbital fissure (Wincza, '98) (figs. 1, 2, 3, 4, 23) in the present stage stands in marked contrast to the circular, completely walled opening of the adult cranium. It is a narrow, deep gap running in a frontal plane between the ala orbitalis above and the ala temporalis and pterygoid cartilage below. It is open laterally, but closed medially by that part of the basis cranii lying between the metoptic root of the ala orbitalis and the processus alaris. It gives passage to the oculomotor, pathetic, ophthalmic and abducent nerves, together with a number of veins which go to join the plexus about the semilunar ganglion and carotid artery.

The carotid foramen (figs. 1, 2) is completely walled, as described by Decker and later by Wincza. It is circular, not large as compared with that in *Lepus*, stands opposite the middle of the sella turcica and traverses the floor of the skull straight ventro-dorsad. In front is the processus alaris; behind, the cochlear capsule; laterally, the alicochlear commissure. Upon its medial side is the sella turcica.



*Pterygoid cartilage* (figs. 1, 3, 24). In the model of the cranium of the 23.1 mm. embryo the pterygoid cartilages appear as a pair of irregular but symmetrically formed bodies lying one on either side of the naso-pharyngeal duct and adjacent to the alae temporales. Each cartilage presents a short cylindrical, transversely placed pedicle and, continuous with it and in a dorso-ventral plane, an elongate, bent plate. The pedicle consists of dense mesenchyma continuous with the perichondrium of the margin of the pterygoid process of the lamina ascendens; in the center bone has begun to form. The N. pterygoideus (Vidiani) turns dorsad upon the posterior aspect of the joint between the pedicle and pterygoid process to enter the spheno-orbital fissure. The plate-shaped part of the pterygoid cartilage presents a flat free surface directed forward, a medial concave margin turned toward the naso-pharyngeal duct and a lateral convex margin giving attachment to the M. pterygoideus. In structure the lamina consists of mesenchymal syncytium, within which bone has formed in two regions. One center lies in the dorsal half of the plate and is continuous with the ossific center of the pedicle; the second is found in the ventral half and seems to be separated from the first by a stratum of unossified tissue, broad at the margin and surfaces of the plate, but becoming narrower toward the center. I find it impossible to state whether the two bony centers so clearly separated one from the other at the margins of the lamina are entirely distinct throughout. A mesenchymal condensation surrounds the pterygoid cartilage, extending forward, becoming somewhat less compact, and finally joining the mass of mesenchyma in which the palate bone is forming. This mesenchymal mantle extends dorsally toward the cranial base without, however, quite reaching it.

The region named *cavum epiptericum* by Gaupp ('05) and interpreted in its relation to the cranial cavity of mammals by this investigator ('02) as an acquisition of a space which, in reptiles, is extracranial, presents some interesting peculiarities in the embryos of cat. First, the epipteric cave with its contained structures is, in the absence of those bones which in the adult form the lateral wall of the skull in this region, wholly outside



the confines of the brain case of the 23.1 mm. embryo. The exclusion of the space from the cavum cranii is brought about by a strong membrane, conspicuous in the sections, which arises from the base of the cranium and is applied to the medial surface of the semilunar ganglion, excluding this structure, as it rests on the ala temporalis, from the cranial space occupied by the brain. Less dense mesenchyma surrounds the ganglion and extends laterally as far as the temporal muscle; forward through the sphenoorbital fissure and about the ala temporalis; backward, into the cavum supracochleare. The medial limiting membrane calls for special description. We may tentatively refer to it as the membrana limitans (figs. 19 to 23). It fills the foramen sphenoparietale attaching itself to its margins, i.e., to the commissura orbito-parietalis, commissura suprafacialis, where it is perforated by the roots of the trigeminus, and to the metoptic root of the ala orbitalis; then upon the base of the cranium, from the anterior pole of the cochlear capsule along the commissura alicochlearis, crista transversa and dorsum sellae, the lateral elevated margins of the hypophyseal fossa, and on to the tuberculum ephippii. In the bottom of the sella turcica and upon the dorsum sellae the membrane is continuous from side to side. At the saddle-back it is continuous with a stout meningeal membrane which ascends into the narrow space in the flexure of the mid-brain and diencephalon; this may be referred to as the septum transversum (see p. 327). The thickness of the limiting membrane is not the same throughout. It is densest at its broad attachment to the cranial base where it covers the carotid foramen. Also a definite band of condensed tissue extends through the septum transversum from the crista transversa, crossing the N. abducens and the roots of the N. trigeminus running parallel with the commissura suprafacialis and reaching laterally the parietal plate. It is along this stretch that a small cartilaginous bar is later developed (embryo of 7 cm.) over the dorso-medial surface of the semilunar ganglion and roots of the trigeminus where the latter cross the suprafacial commissure.

The floor of the epipteric cave is formed by the lamina ascendens of the ala temporalis. The following structures are found

within the cave: the ganglion semilunare and the three divisions of the N. V., the NN. III, IV and VI. The caudal half of the semilunar ganglion rests upon the supracochlear plane, in its cephalic half upon the alicochlear commissure and the posterior subdivision of the dorsal surface of the lamina ascendens. Beneath it, the mandibular nerve passes to leave the cave through the incisura ovalis. The ophthalmic and maxillary nerves leave the region immediately in front of the ganglion, the one by way of the speno-orbital fissure, the other by the foramen rotundum. Both the oculomotor and trochlear nerves run a long course in the loose tissue immediately surrounding the brain, proceeding from their origins ventrad and cephalad toward the speno-orbital fissure, where they lie above the ophthalmic nerve. They pierce the membrana limitans just before entering the fissure, and their course in the epipteric cave is therefore very short (fig. 23). On the contrary, the course of the abducent nerve through the cavum epiptericum is very long; it enters the space from behind, passing between the dorsum sellae and cochlear capsule, over the crista transversa and beneath that strand of condensed mesenchyma in the septum transversum which is the forerunner of the cartilage referred to above. Running cephalo-laterad, it crosses laterally the carotid artery and gains the medial side of the semilunar ganglion and ophthalmic nerve; in the speno-orbital fissure it crosses dorsally the N. maxillaris. The internal carotid artery enters the membrana limitans at the carotid foramen, turns forward in this tissue, crosses the processus alaris at the side of the hypophysis, then pierces the membrane in a dorsal and medial direction to enter the cavum cranii. At its entrance into the basal portion of the membrana limitans the artery is ventrad of the sixth nerve, but it turns immediately mesad beneath it. Several veins accompany the nerves through the speno-orbital fissure and go to form, by anastomoses, a plexus in the membrana limitans which surrounds the carotid artery. The vessels of this plexus are separated by mesenchymal tissue, altogether constituting the beginnings of the channels and walls of the later cavernous sinus.

The orbit (figs. 3, 4, 24, 25, 26) is relatively shallow and is ill-defined in extent and boundaries. The planum antorbitale and prominentia lateralis of the ethmoidal skeleton form its anterior wall; the ala orbitalis and its two commissures enter into the posterior wall. The dorsal limit is given by the frontal bone which extends along the spheno-ethmoidal commissure. The ventral limits are found in the zygomatic and maxillary bones. The ocular muscles arise in the following way. From the pre-optic root springs the superior oblique; from the orbital process of the metoptic root arise the rectus superior, internus and externus. The origin of the inferior rectus is conjoined with that of the retractor oculi on the lateral surface of the cranial base, ventrad of the metoptic root and adjacent processus alaris; these muscles arise within the spheno-orbital fissure. The inferior oblique springs from the ventral margin of the planum antorbitale well forward of the origins of the other ocular muscles.

*Orbito-temporal region in smaller embryos*

Van Wijhe preparations of 10 mm. (fig. 5) present the first trace of chondrification in the orbito-temporal region as a small crescentic cartilage ventrad of the anterior hypophyseal lobe. The concavity of the crescent embraces the hypophyseal stalk. Behind this hypophyseal cartilage and occupying a dorsal plane is the termination of the notochord.

Sections of an embryo of 12 mm. (figs. 16, 17) show that the cranial floor beneath the hypophysis consists of a horseshoe-shaped cartilage independent of other parts of the chondrocranium. The legs of the horseshoe lie on either side of the mid-line beneath the anterior pituitary lobe, reaching as far forward as the trabecular plate, but not united with it; the commissure of the horseshoe crosses behind the stalk of Rathke's pouch. Between the commissure and the terminal expansion of the basal plate is a small space, fenestra basicranialis posterior, occupied by mesenchyma in which the cephalic end of the notochord lies. In median sagittal sections of embryos of 12 mm. (fig. 17) the anterior expanded extremity of the basal plate is turned somewhat in the dorsal direction above the line of the posterior parts

of the hypophyseal cartilage. The trabecular plate makes its appearance in embryos of the present stage (fig. 17), consisting of a single thick mass of young cartilage continuous anteriorly with the beginnings of the septum nasi. Caudally it reaches almost to the hypophyseal cartilage. In the van Wijhe preparations the limits of the trabecular plate are clearer than in the sections (fig. 6). On its dorsal surface the sulcus chiasmaticus appears; on its sides, the beginnings of the pre- and metoptic processes.

In a frontal section passing through the hypophysis and cochlear canal of an embryo of 12 mm. (fig. 16) the carotid artery appears in its course through the cranial wall. It lies in an oval area of less dense mesenchyma than that a little way behind its position. The denser tissue anterior to the area forms a curved bar, continuous with the side of the hypophyseal cartilage medially, thence extending in a curve outside the artery toward the cochlear capsule. This will be referred to as the commissural element; it is the beginning of the processus alaris and commissura alicochlearis. The oval, light area about the carotid is limited caudally and medially by the cochlear capsule and a bridge of mesenchyma (future basicochlear commissure) stretching from the latter to the hypophyseal cartilage.

In the mesenchyma laterad of the commissural element is a small condensation beneath the Gasserian ganglion, which proves to be part of the medial extremity of the future ala temporalis. This condensation will be referred to as the alar element. The less dense mesenchyma between the latter and the commissural element extends obliquely from before, backward and outward. The mesenchyma of the alar element becomes broader and denser as it extends laterally; its caudal concave margin, pressed against the mandibular nerve, forms the primitive incisura ovalis; within its anterior part appears the maxillary nerve. The ophthalmic nerve (whose ganglionic cells are in part separate from the semilunar ganglion) passes over the alar element toward the eye. The van Wijhe specimens of 12 mm. (fig. 6) show a small darkly stained nodule far removed from the hypophyseal cartilages and lying immediately ventrad of the anterior end of the semilunar



ganglion. This center is located at the site of the anterior part of the alar element.

In sections the ala orbitalis is a plate of condensed mesenchyma limited sharply in front, above and behind, but connected with the trabecular plate by two roots. The preoptic root extends from the trabecular plate anterior to the sulcus chiasmaticus and passes laterally without interruption into the main body of the ala. The metoptic root is joined to the side of the caudal portion of the trabecular plate. This root terminates laterally in a pointed extremity connected by less dense mesenchyma with the ala orbitalis. The extremity lies beyond the optic foramen, immediately dorsad of the third nerve where this enters the orbit.

The cavum epiptericum in the 12 mm. embryo, is clearly defined toward the base of the neuro-cranium. The membrana limitans (figs. 16, 17) can be followed over the medial aspect of the semilunar ganglion to the anterior end of the basal plate, to the commissural element and over the carotid artery. Upon the lateral side of the membrane, opposite the vessel runs the abducent nerve. At its insertion into the anterior margin of the basal plate (the future crista transversa) the limiting membrane becomes continuous with the septum transversum. Within the latter immediately caudad of the hypophysis is a small, transversely placed precartilaginous rod (indicated by an \* in fig. 17).

The pterygoid cartilage is represented by a condensation of mesenchyma lying anterior and ventral of the alar element and close to the epithelium of the pharynx. A constriction incompletely subdivides it into dorsal and ventral parts. Behind this mass is the first pharyngeal pouch; through its dorsal extremity runs the pterygoid nerve.

In embryos of 15 mm. the hypophyseal cartilage has united anteriorly with the trabecular plate, forming the floor of the sella turcica, perforated by a foramen hypophyseos (figs. 7, 18). Union of the basal plate and hypophyseal cartilage has also occurred, but the confines of the originally separate cartilages are still evident (fig. 18). As a result of the incongruity in the meeting of these parts (indicated in the 12 mm. stage) there remains a transverse ridge in the basis cranii behind the pituitary fossa,



the beginning of the crista transversa. This is the stage of precartilaginous structure of the alar and commissural elements. The latter is continuous with the side of the sella turcica and separated from the alar element by an oblique layer of mesenchyma. The boundaries of the carotid foramen are well defined, the medial boundary being formed by the posterior part of the side of the sella turcica; the caudal limit by the prochondral union of the basal plate and cochlear capsule, the commissura basicochlearis; the anterior and lateral by the prochondral commissural element. The alar element has a homogeneous structure of young cartilage and is now recognizable as the ala temporalis; both the mandibular and maxillary nerves pass through notches in its margins.

In van Wijhe preparations of 15 mm. (fig. 7) a continuous stretch of cartilage occurs in the floor of the cranium in the otic, hypophyseal, trabecular and nasal septal regions. The tuberculum sellae is apparently developed from the caudal and dorsal edge of the trabecular plate. The junction of the basal plate and floor, in the hypophyseal region, is marked by a broad expansion of lightly stained tissue (young cartilage). In a median section (fig. 18) the cartilage in the septum transversum behind the hypophysis, present in the preceding stage, appears as a small nodule. The ala temporalis is represented by a slender sickle-shaped cartilage standing ventrad of the anterior pole of the semilunar ganglion. It is laterally removed from the sella turcica and from the commissural element (the interval is exaggerated in figure 7). In the epipteric region the membrana limitans is clearly defined throughout. The origin of the transverse septum is marked by a band-like thickening, extending from the crista transversa laterad over the semilunar ganglion and roots of the trigeminus, and terminating on the suprafacial commissure and parietal plate.

The pterygoid presents no important change from the preceding stage. Chondrification of the ala orbitalis has begun over the eye-ball in the form of a triangular plate, separate from all neighboring cartilages (fig. 7). Posterior to the orbital ala is the independently chondrifying commissura orbito-parietalis; far removed anteriorly are the cartilages of the nasal capsule. One

angle of the ala orbitalis is directed medially toward the trabecular plate and its two processes, preoptic and metoptic. The extremity of the latter presents the relation with the oculomotor nerve observed in the earlier stage. From the metoptic process a thick projection extends a short distance laterally, just behind the optic nerve, to end in the muscle mass about the nerve; this is the beginning of the orbital process of later stages.

Van Wijhe preparations of embryos of 17 mm. (fig. 8) show a lightly stained curved bar, the combined processus alaris and aliochlear commissure, extending around the carotid artery, from the side of the sella turcica to the anterior pole of the cochlear capsule. Just in front and laterad of the commissure, and separated by an interval of unstained tissue, is the triangular ala temporalis, with the maxillary nerve against its anterior, concave margin, and the mandibular nerve at its posterior side. The separate cartilage behind the infundibulum in the preceding stage is no longer apparent in the sections, but there is now at this spot a dorsal, median process, partly cartilaginous, largely mesenchymal, of the crista transversa. Union of the orbital plate with neighboring skeletal parts has occurred (fig. 8): the medial angle, presenting a notch for the optic nerve, is connected with the pre- and metoptic processes of the trabecular plate; the anterior angle is prolonged to the paranasal cartilage as the commissura spheno-ethmoidalis, thereby completing the boundaries of the fissura orbito-nasalis; the posterior angle is fused with the commissura orbito-parietalis. This commissure, which has united also with the parietal plate, has extended ventrally to the pars canalicularis (commissura parieto-capsularis), but, as sections show, is separated from the otic capsule by a thin layer of mesenchyma (p. 313). Continuity of the cartilage of the orbito-parietal commissure and suprafacial commissure has been described above (p. 301).

*Ethmoidal region. Embryo of 23.1 mm..*

The parts of the chondrocranium included in this most clearly defined region of the skull are the paired nasal capsules (figs. 1, 2, 3, 4). These conform closely with the membranous walls of

the cavum nasi within, and are shaped externally somewhat like a pair of spindles pressed together, making a single strong framework, the cartilaginous ethmoidal skeleton, at the anterior end of the skull.

The length of the cartilaginous ethmoidal skeleton is equal to about one-third that of the entire cranium. Its anterior half projects free beyond the brain case; its posterior half, terminating on each side in the posterior cupola, is subcerebral in position. Here it is continuous with parts in the orbito-temporal region: the trabecular plate passing into the septum nasi and roof of the posterior cupola, the commissura spheno-ethmoidalis joining the lateral nasal wall. An extensive roof, tectum nasi, is in the anterior half of the ethmoidal skeleton; an incomplete one, lamina infracribrosa, in the posterior. The roof goes over on either side into the paries nasi. The interior room of the nasal skeleton opens into the cranial cavity by the fenestrae olfactoriae, and upon the face through the fenestrae narinae. The floor of the capsule, solum nasi, is very incomplete, presenting a great fenestra basalis, within the compass of which are the paraseptal cartilages standing next to the nasal septum. The side walls within, present the beginnings of the complex of turbinals in the form of simple inrollings and elevations.

The tectum nasi (figs. 1, 3, 27 to 30) is furrowed in the mid-line of its dorsal surface by the sulcus suprasedalis, deepest anteriorly, extending from the fenestra olfactoria to the level of the fenestra narina. On either side the roof is highly arched and passes laterally into the anterior region of the paries nasi.

The side wall (figs. 3, 4) presents three regions: an anterior, continuous with the tectum nasi, a middle region occupied by the rounded prominentia lateralis, and a posterior area, the planum antorbitale. Two grooves demarcate the prominentia lateralis from the anterior and posterior regions. One of them, the sulcus lateralis anterior, at first pursues a curved course from the olfactory window near the mid-line, cephalo-ventrad between the tectum nasi and prominentia lateralis. This part of the sulcus stands opposite the crista semicircularis within the nasal capsule and is interrupted by the epiphanyal foramina of which

there are two on each side. In its ventral one-third the groove is broad and shallow and adjoins the anterior region of the paries nasi and the lateral prominence. It occupies an area which passes ventrally into the maxilloturbinal and stands at a level anterior to the crista semicircularis. This part of the paries nasi corresponds to the lamina supraconchalis of Voit. The second groove, sulcus lateralis posterior, limits the lateral prominence posteriorly; it is broad and shallow and stands opposite ethmoturbinal I within the capsule. The anterior region of the paries nasi extends from the antero-lateral sulcus to the lateral margin of the fenestra narina. It is co-extensive with the roof dorsally, but becomes narrow ventrally where it joins the lamina transversalis anterior. The surface in this region is convex, corresponding to the internal concavity of this part of the lateral wall. The middle region, the prominentia lateralis, is narrow from before backward. Dorsally it is united with the commissura spheno-ethmoidalis and forms the lateral boundary of the olfactory window, between the posterior extremities of the crista semicircularis and ethmoturbinal I (vide infra). Ventrally it passes into the maxillo-turbinal. The posterior area, antorbital plane, corresponds to the region within occupied by the ethmoturbinals, and forms the antero-medial wall of the orbit. Where it adjoins the middle region of the paries nasi (sulcus lateralis posterior) there is present ventrally a rounded prominence, giving origin to the inferior oblique muscle of the eye. The dorsal margin of the antorbital plane at the orbito-nasal fissure, participates, anteriorly in the lateral boundary of the olfactory window, and posteriorly passes into the lamina infracribrosa. The ventral margin is straight and free in the lateral boundary of the fenestra basalis. Caudally, this division of the nasal capsular wall continues into the posterior cupola. This conical shell extends backward at the side of the nasal septum and trabecular plate beneath the preoptic root of the ala orbitalis. It is closed ventrally by the lamina transversalis posterior, which reaches to the septum nasi. The roof of the posterior cupola is formed by the unpaired lamina infracribrosa (figs. 1, 25). This plate presents a free edge anteriorly toward the fenestra olfactoria, con-



tinues caudally into the dorsal surface of the trabecular plate and laterally goes into the planum antorbitale. A slight median ridge, crista interorbitalis, separates two shallow fossae lodging the olfactory tracts.

Ventrally, the nasal capsule presents the great fenestra basalis (figs. 1, 2), the relations of which to the cavum nasi are somewhat complicated. It is limited laterally by the ventral margin of the planum antorbitale and by the maxillo-turbinal. In the region of the latter it is very narrow from side to side, and is partly covered ventrally by the paraseptal cartilage hanging down from the septum. Anteriorly the basal window reaches to the lamina transversalis anterior which stands between it and the fenestra narina. The caudal limit is formed by the lamina transversalis posterior. Further relations of the basal window will be better understood when considered in connection with the description of the nasal cavity.

The solum nasi is represented by the two transverse laminae and the horizontal portion of the paraseptal cartilage (figs. 2, 3, 13, 25, 26). The lamina transversalis posterior (figs. 2, 13, 25) forms the floor of the posterior cupola and appears in the sections and in the model as an inrolling of the caudo-ventral part of the planum antorbitale. Its medial margin is turned dorsad; in its caudal half it is applied closely to, but not actually joined with, the keeled trabecular plate behind the level of the vomer; in its cephalic half it is free, being separated from the nasal septum by a narrow stretch of the fenestra basalis. Here the lamina is overlapped by the caudal end of the vomer. The band-like lamina transversalis anterior (figs. 2, 3, 13), incompletely chondrified, bounds the fenestra narina ventro-laterally, connecting the septum nasi with the paries nasi. It is continuous with both parts, that is to say, a zona annularis is present. Its horizontal, broader, septal end stands opposite the anterior half of the os incisivum. Followed laterally, the lamina undergoes a curve in the dorsal direction, and comes to lie in a sagittal plane.<sup>3</sup>

<sup>3</sup> In cat embryos of about 70 mm. the lamina transversalis anterior presents two backwardly directed processes which embrace the funnel-shaped depression of the floor leading into the incisive duct. These have been called by Zucker-



Just ventrad of its junction with the anterior part of the paries nasi it is crossed by the naso-lacrimal duct. The caudal margin of the lamina is free and is separated by a wide interval from the paraseptal cartilage.

Behind the anterior transverse lamina, the tear duct lies upon the most ventral part of the paries nasi and is further supported by a cylindrical processus maxillaris anterior of the latter (figs. 3, 13, 30) directed forward. At this place the os maxillare overlaps the nasal capsule and, in the model, hides from view the maxillary process and tear duct.

The cartilago paraseptalis (figs. 2, 3, 13, 28, 29), scroll-formed in adaptation to Jacobson's organ, hangs from the septum nasi below the level of the plane of the fenestra basalis. It is unconnected with other parts of the nasal skeleton. The medial half of each cartilage lies in a sagittal plane applied against the ventral part of the septum. The lateral half is rolled dorsally to form a gutter lodging the vomero-nasal organ. The cephalic margin is free and straight; the caudal pointed. The long axis of Jacobson's cartilage extends from behind cephalo-ventrad, the caudal extremity lying at the level of the basal window, the cephalic end reaching a position ventrad of the plane of the anterior transverse lamina. At this stage the paired naso-palatine cartilage is represented by a stretch of prochondral tissue upon the lateral side of each incisive duct.<sup>4</sup> The U-shaped beginning

kanal the medial and lateral limbs of the ventro-lateral process of the septal cartilage. The incisive duct is partly enveloped on its lateral side by a trough-shaped nasopalatine cartilage, which reaches from a spot near the oral end of the duct, to the level of the entrance of the duct of Jacobson's organ. The last named canal is surrounded completely by a cartilaginous tube which, as it ascends toward the cavum nasi, gradually loses its dorsal wall and becomes continuous with the anterior extremity of the paraseptal (Jacobson's) cartilage. The cartilage of Jacobson's duct lies medial of the process of the lamina transversalis anterior; neither it nor the nasopalatine cartilage is joined with the processes of the anterior transverse lamina.

<sup>4</sup> The paraseptal cartilage in embryos of 70 mm. presents an anterior process, continuous with the cartilage of the duct of Jacobson's organ, and continues posteriorly into a slender cartilaginous paraseptal rod. The latter lies against the side of the ventral edge of the vomer and extends to the floor of the posterior cupola, the lamina transversalis posterior with which it is directly continuous.

of the vomer (figs. 2, 26, 27) embracing the ventral edge of the septum nasi, lies at some distance behind the paraseptal.

The fenestra narina (figs. 2, 3, 4, 13), oval with its long axis dorso-ventral, faces rostro-laterad. Its medial boundary is formed by the septum, the lateral and ventral by the lamina transversalis anterior, the dorsal, by the paries nasi. The lateral margin presents a deep notch, the incisura lacrimalis, where the naso-lacrimal duct turns medialward toward its termination. The ventral margin of this notch is formed by the sagittal portion of the anterior transverse lamina; the dorsal margin by the rostral extremity of the atrioturbine, or inrolled ventral margin of the paries nasi. The lacrimal notch is the meeting place of two divisions of the fenestra narina, a dorsal and a ventral. The former leads to the atrial region of the cavum nasi; the latter division receives the naso-lacrimal duct and opens into the inferior meatus. The names pars atrialis and pars lacrimalis, respectively will be used in further reference to these divisions of the fenestra narina. In the present stage the superior alar process is represented in dense mesenchyma. It is chondrified in the 7 cm. embryo.

*Fenestra olfactoria* (figs. 1, 13, 26). The paired, quadrilateral olfactory fenestrae offer wide communications, in the present stage, between the nasal and cranial cavities. Their position is in the caudal half of the nasal capsules, that is, in the subcerebral division, between the tectum nasi, anteriorly, and the roof (lamina infracribrosa) of the posterior cupola, posteriorly. The plane of each opening looks dorso-caudad and also somewhat mesad. The anterior boundary, formed by the caudal free margins of the tectum nasi and prominentia lateralis, is considerably wider than the posterior limit, made by the infracribrous plate. The medial side is formed by the dorsal concave margin of the septum nasi. The oblique lateral boundaries of the olfactory fenestrae are formed by the dorsal margins of the prominentiae laterales and the antorbital plates. On the anterior and lateral sides of the fenestra olfactoria of the model are three processes, the caudal extremities of the crista semicircularis, ethmoturbinal I and ethmoturbinal II. Between these processes, the

olfactory fenestra extends in the form of three bays or sinuses. In the present stage there is no cartilaginous cribriform plate; its place is occupied by mesenchyma perforated by the olfactory nerves. These are in two groups, one near the septum, the second represented by bundles passing through the sinuses at the side of the window. The sheet of mesenchyma perforated by the olfactory nerves does not quite fill the fenestra olfactoria. It is closely applied to the septum nasi, then crosses the mid-line to be continuous with the membrane of the opposite side; it is fixed at the anterior boundary of the window (tectum nasi). Posteriorly the membrane passes over the lamina infracribrosa against which it is closely applied. In the lateral direction it becomes continuous with the membrane filling the spheno-ethmoidal fissure. This is fixed to the dorsal but not to the ventral boundary of the fenestra, which, it will be recalled, is formed by the antorbital plate. In relation to this membrane is the lateral branch of the nasal nerve (fig. 26). The latter appears in the sections through the anterior part of the orbit, running toward the spheno-ethmoidal fissure. This it enters, passing ventrad of the membrane, a position it retains in its course forward to the olfactory fenestra. Here it enters the nasal capsule, pursues a short course through the crista semicircularis and then passes to the exterior by one of the epiphantal foramina.

*Septum nasi* (figs. 1, 2, 25 to 30). This median partition is the direct continuation of the trabecular plate forward into the ethmoidal region. It is low and broad caudally where it lies between, and enters into the median walls of the posterior cupolae. As it extends forward the dorsal margin ascends, the height of the septum increasing gradually toward the fenestrae olfactoriae; between the olfactory fenestrae it increases rapidly and reaches its maximum height at the tectum nasi; then decreases gradually in approaching the level of the fenestrae narinae. The ventral margin of the septum presents a longitudinal concavity, and is thickened between the paraseptal cartilages and again opposite the vomer. Anterior to the paraseptals it is united on each side to the anterior transverse lam-

ina, the line of junction being marked ventrally by a longitudinal groove. The anterior free margin is straight and reaches further rostrad than the lateral wall of the nose. Dorsally, the septum presents between the olfactory fenestrae a free concave edge (there is no evidence of a crista galli) and, in its precerebral portion, continues into the tectum nasi. The latter relation appears in the sections of the anterior third of the nose as a bifurcation of the septum into two laminae, which extend on either side into the roof of the nose (fig. 28). In this way the sulcus suprasedentalis is formed.

*Interior of the nasal capsule* (figs. 13, 25 to 30). The conformation of the nasal capsular walls from within is extremely simple at the present stage as compared with that of the adult. Turbinal processes are in evidence in the form of ridges, the maxilloturbinal alone showing any degree of scroll form. The bones in relation to the nasal cavity are the vomer, incisum and maxilla.

*Lateral wall of the cavum nasi.* In figure 13, which represents the left lateral wall of the nasal capsule from within, the following parts, already referred to, will be recognized: the tectum nasi extending from the fenestra narina to the fenestra olfactoria, the posterior cupola and lamina transversalis anterior, parts showing cut surfaces at their junction with the septum nasi; ethmoturbinal I and II presenting at the olfactory fenestra; the crista semicircularis and the nasoturbinal; the inrolled ventral margin of the paries nasi forming the maxilloturbinal and atrioturbinal. The lateral wall of the cavum nasi presents two unequal divisions: a small region ventrad of the level of the maxillo- and atrioturbinals, and an extensive region dorsad of this level. The former includes the sagittal portion of the lamina transversalis anterior and the incisura posttransversalis; the latter corresponds to the rest of the lateral nasal wall.

*Ventral region of lateral wall of the cavum nasi.* The sagittal portion of the lamina transversalis anterior presents a smooth, concave surface toward the cavum nasi, becoming broad ventrally where it passes into the floor of the nose (frontal portion of the lamina transversalis anterior). Caudad of the lamina



the cartilaginous lateral wall of this region is deficient; here the great notch, the incisura posttransversalis is found (figs. 2, 3, 4, 13, 29). This space is entered from behind and ventrally by the cartilaginous anterior maxillary process. The post-transverse notch is closed toward the nasal mucosa, by a layer of mesenchyma and by the incisive and maxillary bones which lie just outside the plane of the cartilaginous nasal wall (figs. 29, 30).

*The dorsal region of the lateral wall of the cavum nasi* corresponds on the exterior to the paries nasi as described on p. 380 and may, like it, be considered as presenting three divisions, anterior, middle and posterior.

In the anterior division (*pars maxillo-nasoturbinalis*) will be included, for convenience, the inner surface of the lamina supraconchalis, although, as will be seen later, its primary relation to the components of the nasal side wall was not established. This division, then, extends from the level of the fenestra narina to the crista semicircularis, and is limited ventrally by the maxillo- and atrioturbinals. The most cephalic portion reaches the atrioturbinal ventrally, and forms the lateral boundary of that part of the nasal cavity here designated the atrium. The atrioturbinal is in line with the maxilloturbinal but is separated from that process by a notch, the incisura maxillo-atrioturbinalis. The succeeding part of the anterior division stands opposite the area of the lamina supraconchalis and sulcus lateralis of the external surface of the nasal capsule, and further includes the maxillo-turbinal and crista semicircularis in its ventral and caudal limits respectively. The maxilloturbinal, triangular in general form, is continuous laterally by its base, with the paries nasi at the eminentia lateralis. It stands mainly in a frontal plane; its medial margin, opposite the septum, inclines ventro-caudad to meet the caudal margin at an angle opposite the paraseptal cartilage. Anterior to the level of the paraseptal it participates with the paries nasi in the caudal boundary of the post-transverse notch; posteriorly to the paraseptal it forms, by its free margin, a wide notch or sinus with the ventral free border of the anteorbital part of the paries nasi, both entering into the boundary of the fenestra basalis. The maxilloturbinal



reaches its greatest breadth opposite the base of the crista semicircularis; from this point caudad it rapidly diminishes in width. On the lateral wall, dorsad of the maxilloturbinal, is a low antero-posterior eminence produced by a slight inward bulging of the paries nasi in the region of the lamina supraconchalis. This elevation is at the base of the great naso-turbinal body composed of mesenchyma in the present stage (figs. 13, 29). Posteriorly, the elevation in question and the nasoturbinal continue into the crista semicircularis (figs. 13, 27, 28, 29). The latter, at present, forms the anterior boundary of the entrance into the recessus lateralis (figs. 23, 27, 28, 29); it extends ventro-dorsad to the tectum nasi, sweeping in a fine curve caudally, alongside the septum, and terminating at the olfactory fenestra as already described (p. 334, fig. 1). Where the crest approaches the tectum nasi, more or less complete discontinuity of the cartilage occurs in the line of the sulcus lateralis. A small fissure here, made by the foramina epiphania (figs. 28, 29), separates, for a short distance, the wall of the recessus lateralis from the tectum nasi. A wide meatus of the lateral nasal wall (here termed meatus supraconchalis) runs between the naso- and maxilloturbinal bodies, continuing anteriorly into the atrium. Posteriorly this meatus extends ventrad of the crista semicircularis (here it is very narrow) and enters into a wide space which opens into the fenestra basalis (fig. 13). Just where the meatus passes into this space, and at a spot anterior to the ventral end of the crista semicircularis, the lateral nasal glands lie under cover of the mucosa (fig. 27). In its posterior half the meatus supraconchalis is extended into a groove between the maxilloturbinal ventrally, and paries nasi (lamina supraconchalis) laterally, the sulcus supraconchalis of Voit.

The middle division corresponds to the prominentia lateralis of the exterior and presents the recessus lateralis to which reference has been made. This large cavity communicates with the general room of the nasal capsule by a wide opening behind the crista semicircularis. Anteriorly it undermines the crest for a short distance in the form of a blind pocket; caudally it reaches the first ethmoturbinal, and dorso-caudally opens into

the olfactory fenestra through the sinus between the crista semicircularis and ethmoturbinal I (p. 335). Although at this stage the cartilaginous parts fail to meet in forming a medial wall for the inferior portion of the lateral recess, the soft parts which stretch between ethmoidal I, the crista semicircularis and the floor shut off this part of the recess from the common meatus of the nose and convert this part of the cavity into a blind pocket. Similarly, the entrance from the common meatus into the recessus lateralis between ethmoidal I and crista semicircularis is very broad, as shown in the models, but by the presence of the soft parts over these elevations, it is reduced to a narrow fissure, the hiatus semilunaris of later stages. The lateral recess is incompletely subdivided into dorsal and ventral rooms, recessus lateralis superior and recessus lateralis inferior, by a slight frontal ridge springing from the lateral surface of ethmoturbinal I, and by an oppositely placed ridge of the lateral wall of the recess (fig. 27). The superior lateral recess opens into the common meatus of the nose through the dorsal part of the hiatus semilunaris. Within it are two antero-posterior, curving ridges of the mucosa, one upon the roof, the other on the lateral wall. Each includes a condensation of the mesenchyma which is in contact, but apparently not continuous by any transitional zone, with the cartilaginous wall. These ridges separate three out-pocketings of the recessus lateralis superior, a lateral, a superior and an inferior groove (fig. 27). The recessus lateralis inferior is incompletely walled medially, as already explained. It lodges a blind sac of mucosa which communicates with the common meatus through the ventral part of the hiatus semilunaris.

*Posterior division.* (Pars ethmoturbinalis). This region of the dorsal part of the lateral nasal wall corresponds to the antorbital plane of the exterior. It is characterized by the presence of the bases of the ethmoturbinals. Ethmoturbinal I appears in the model as a massive irregular ridge, extending dorso-ventrally on the lateral nasal wall at the caudal margin of the entrance into the recessus lateralis (fig. 13). Anteriorly, the process goes over into precartilage in the form of a broad,

thick piece, triangular in section, extending cephalad toward the crista semicircularis (fig. 27). Its medial surface bounds the common meatus; a dorso-lateral surface enters into the floor of the recessus lateralis superior; the ventro-lateral face is turned toward the recessus lateralis inferior. Ethmoturbinal I reaches the olfactory fenestra in the thick layer of mesenchyma filling this space (fig. 1); ventrally it is separated by a wide stretch from the level of the solum nasi; anteriorly it enters into the caudal boundary of the hiatus semilunaris. Ethmoturbinal II (figs. 1, 13, 26) is represented by a small cartilaginous plate, with precartilaginous margins, jutting mesad from the lateral nasal wall between ethmoturbinal I and the posterior cupola. From the level of the olfactory fenestra its long axis extends ventro-rostrad. It terminates a considerable distance above the plane of the fenestra basalis.

The cavity of the posterior cupola lies behind ethmoturbinal I, occupying the caudal extremity of the nasal capsule. The walls formed by the septum nasi, lamina infracribrosa, lamina transversalis posterior and antorbital plane, are smooth. The cavity opens anteriorly and ventrally into the common meatus of the nose and the fenestra basalis.

#### *Ethmoidal region in smaller embryos*

The first evidences of chondrification in the ethmoidal region were found in embryos of 12 mm. in which the process was manifested in the septum nasi (fig. 17). In van Wijhe preparations the ventral part of the septum was stained blue (fig. 6), appearing in the form of two streaks extending from the trabecular plate forward, side by side and separated by a less deeply stained tract. In embryos of 15 mm. chondrification of the septum has extended dorsally, reaching its greatest height in front. It is now a single median cartilage (fig. 18). The septum is continuous with the trabecular plate caudally, and in front gives off a pair of arching processes from its dorsal margin (fig. 7). The latter, which may be called the parieto-tectal cartilages, are at this stage in relation to the roof and lateral wall of the anterior one-third of the nasal cavity. Whether these processes are pri-

marily outgrowths of the septum is brought into question by the fact that they are most deeply stained in the van Wijhe preparations in their lateral parts and less so next to the septum. In the midline dorsally a deep groove, the beginning of the sulcus supraseptalis, lies between them. Besides the paired parietotectal cartilages, there is, in the nasal region of van Wijhe preparations of 15 mm., a mass of cartilage quite independent of other chondrifying tracts. This is a curved plate, overlying, on each side, the diverticulum of the *cavum nasi*, which later is included in the recessus lateralis of the cartilaginous wall. This cartilage may be referred to, tentatively, as the paranasal cartilage. The parietotectal and paranasal cartilages stand close together, the one in front of the other. It is of importance to note that the dorso-cephalic margin of the latter overlaps the dorso-caudal edge of the former. Van Wijhe preparations of 17 mm. exhibit still a third chondrifying tract in relation to the nasal wall (fig. 8). This is a small plate of cartilage at the very back of the *cavum nasi*, on either side of the nasal septum. It appears to be entirely free from the septum and paranasal cartilage. This lamina antorbitalis, as it may be called, lies in a plane anterior to that of the origin of the preoptic root from the trabecular plate, and behind the paranasal cartilage. It is curved about the caudal end of the nasal sac, thus indicating the beginning of the posterior cupola. The anterior margin of the antorbital lamina projects into the fold of ethmoturbinal I behind the diverticulum of the lateral recess, and is overlapped by the caudal edge of the paranasal cartilage. The latter is larger than in the preceding stage and presents anterior and ventral incurved margins, continuous with each other. The ventral edge projects into the fold of the maxilloturbinal and represents the base of the cartilaginous process of that name. The anterior incurved margin overlaps the lateral and, at the same time, caudal margin of the parietotectal, and fusion has occurred to some extent between them. A double layered curved ridge is thus formed, projecting into that fold of the nasal wall which bounds the lateral recess anteriorly; this is the beginning of the crista semicircularis. Where fusion has not occurred be-

tween the paranasal and parieto-ectal cartilage, spaces remain, one of which is traversed by the lateral branch of the nasal nerve; this is the beginning of the foramen epiphaniale. The overlapping and fusion in the ventral region occurs between the inrolled anterior and ventral margins, where, in later stages, the lamina supraconchalis and nasoturbinal are found. The formation of these parts, however, was not observed. The dorsal margin of the parieto-ectal cartilage lies next the olfactory bulb and is joined with the commissura spheuo-ethmoidalis. The triangular parieto-ectal cartilage has grown backward along the septum nasi nearly its full length, thus forming the roof of the nose. Its caudal and lateral oblique margin enters into the formation of the crista semicircularis. In its anterior part it reaches, in a ventral direction, as far as the naso-lacrimal duct. The lamina transversalis anterior is unchondrified. The paraseptal cartilages are represented by incompletely chondrified tracts, independent of other skeletal parts.

## PART II. DISCUSSION

### *Occipital region*

*Basal plate.* As we have seen, the first evidence of a basal plate was in the form of a pair of small cartilages on either side of the notochord of the occipital region, united anteriorly by a hypochordal commissure. Wincza has already observed this early form of the base of the cranium and called the two component laminae the parachordal plates.

Regarding the chondrification of the basal plate in mammals, several authors have shown that the initial stage is characterized by the presence of a pair of cartilaginous centers or tracts, one on either side of the notochord in the occipital region. Parker refers to the basal chondrification in the embryo pig, first ('75), as the 'investing mass,' later ('77) as the parachordal



cartilages. It is not clear from the description that there are two separate elements present. The term "investing mass" is misleading with respect to the notochord; Parker expressly states that the former lies beneath the notochord and so represents the relations of these two parts in the figures. Froriep ('86) recognized a tendency to the formation of bilateral symmetrical anlagen of the caudal part of the occipital floor in the calf (p. 91, woodcut to fig. IV, fig. IV, 2). This author remarks on the striking difference between cervical vertebrae and the occipital vertebra presented by the bilaterality of the anlagen of the bodies. In the former it becomes more pronounced in the cranial direction, whereas in the occipital vertebra this condition is presented in a lesser degree. In reference to the anlage in the occipital floor, Froriep says: "In einem Querschnitt dagegen wie Fig. IV, 2, ist eine bilaterale Sonderung des Knorpelgewebes nicht zu bemerken, die Knorpellage ist hier ventralwärts der Chorda fast ebenso mächtig wie zu beiden Seiten" (p. 91).

In the light of recent studies the condition represented in the figure might justly receive a different interpretation with respect to bilaterality of the anlage; the figure shows two cartilages in the occipital floor, one on each side of the notochord and some distance removed from it, united by continuous cartilage across the midline ventrad of the notochord. Levi ('00) described a pair of precartilaginous and cartilaginous anlagen for the middle piece (basilar portion) of the occipital region in a human embryo of about 13 mm. These were united across the midplane by connective tissue in which ran the notochord, and which was continuous in a caudal direction with the connective tissue of the first cervical vertebral arch (p. 355). Bardeen ('08) also observed the beginning chondrification of the base of the occipital in man in two bilateral centers. Other investigators have recorded an unpaired chondrogenous beginning of the occipital basal plate, or, again, its development in connection with the lateral occipital arch. Weiss ('01) found, in the white rat, that chondrification of the floor of the occipital region was manifested, first, by centers in the hypochordal region, one of them in Froriep's apparently unsegmented portion, the other in the

region of Froriep's occipital vertebra. Noordenbos ('05) found the occipital basal anlage to be single in the mole, calf and pig and also in the rabbit. Regarding the parachordal plate in the latter, this author found that it was formed by the fusion of the opposed ends of the two free occipital arches which were first to develop and from this starting point, grew forward (p. 375). Here are then, apparently, three different conditions presented in the origin of the cartilaginous basal part of the occipital in mammals: the appearance of a hypochordal center; a pair of bilaterally placed masses; origin by growth and fusion of the apposed ends of the lateral occipital arches.

Regarding the relation of the notochord to basal plate, it was found that in cat embryos of about 10 mm. the former enters the occipital region between the parachordal cartilages and lies in the dorsal part of the mesenchymal sheet which unites these cartilages across the midplane. This mesenchymal sheet later becomes chondrified in connection with the parachordals, forming thus a hypochordal bridge closing the space which originally separated these cartilages (Terry, '13). Where the notochord lies between the parachordals it is surrounded by a layer of denser mesenchyma than that concerned in hypochordal arch formation; this specialized sheath is continuous with the mesenchyma about the notochord in the region of the atlas—that in which the atlantal centrum is later formed. In regard to a cartilaginous hypochordal layer in the occipital region of mammals generally, there seems to be no doubt of its constant occurrence. My own observation of the position of the notochord with reference to the basi-occipital region in cat is in agreement with that of Williams ('08), namely, that after chondrification is well established, the caudal portion of the basal plate is hypochordal. Recently Kernan ('15) has observed the hypochordal position of the basal plate in cat embryos. Parker, Froriep, Levi, Noordenbos, and Weiss have observed, in various mammals, that the caudal part of the occipital basal cartilage is ventrad of the notochord.

The parachordals in cat are united at their anterior ends across the midplane (embryos of about 10 mm.) by a hypo-

chordal commissure of young cartilage. This lies at a level cephalad of that of the anterior root bundle of the hypoglossal nerve. Cartilage afterwards forms dorsad of the notochord in the region of the primary commissure, so that the former comes secondarily to lie within the basal plate. I am aware of the fact that some observers have described this anterior commissure of the parachordal in other forms as lying dorsad of the notochord, but in cat I have found it primarily hypochordal. Weiss, however, found the primary process of cartilage formation hypochordal in the apparently unsegmented division of the basal plate. The next cartilaginous union to be established between the parachordal cartilages in cat is the hypochordal arch uniting their caudal extremities. This has formed somewhat earlier than the anterior arch of the atlas, next to which it stands. The posterior hypochordal commissure is in the same transverse plane as the primitive lateral occipital arch. Between the two hypochordal arches, anterior (primary) and posterior, there remains a sheet of mesenchyma, stretching from side to side beneath the notochord, and continuing into the medial edges of the parachordals. The direction in which chondrification proceeds in this tissue is lateromesad. In the middle of this tissue a third commissural process is indicated where the medial edge of each parachordal sends a projection toward the mid-plane, producing a constriction in the vacuity between the parachordals. These two symmetrical processes are directed ventro-mesad to a hypochordal plane and lie at a level corresponding to the middle of the future hypoglossal foramen. In the smaller embryos (9 mm.) four membranous arches in the occipital region were noted by Kernan, who states that the two cranial have a tendency to fuse.

When, in the cat, the parachordal plates have formed, the three root bundles of the hypoglossal lie against their lateral margins, in the angle formed by the lateral occipital arch. The foramen is later completed by the formation of a cartilaginous bar in front of the nerve roots, which extends laterally and dorsally from the anterolateral corner of the parachordal, to unite with the primary lateral occipital arch beyond the

nerve roots. The three roots are united in passing through the hypoglossal notch of the primitive cartilaginous occipital element, and there is no indication of a subdivision of the margin into smaller notches for each root, as appears to be the case in an earlier stage and before cartilage has formed. This condition varies in mammals; in rabbits for example, (Noordenbos, Voit) two canals are present on each side. That two canals occur occasionally in the human skull as an anomaly is well known. It will be recalled that the hypoglossal nerve in cat embryos shows a tendency to conform with a spinal nerve in presenting a posterior root and ganglion in relation to the third motor root. The presence of this ganglion was discovered by Vulpian ('62) and I can confirm this observation here.

The presence of more than two cartilaginous hypochondral commissures in the development of the occipital basal plate has, I believe, not been recognized. Also, the recognition of the primary hypochondral nature of the anterior commissure apparently rests on only one other observation, that of Weiss, in the rat. Until further distribution of these phenomena be discovered, or the present observation confirmed, it would seem of little profit to attempt an interpretation of their significance. The antero-posterior succession of a series of transversely placed bar-like structures in the base of the skull at once suggests the idea of segmentation. A relation of these bars laterally with the hypoglossal nerve roots may or may not be a primary one. But in utilizing the results of chondrocranial study in any discussion of the segmentation of the cranium, it must be borne in mind that such evidence can be of high value only when the relation of chondral to blastemal developmental processes is known. It seems highly probable that the cartilaginous commissures here described and the membranous bars observed by Kernan ('15) are two developmental phases of one and the same process, which subsequent investigation will elucidate.

In regard to the condensed tissue sheath about the occipital notochord found in cat, it may be said that a number of observations made on different animals point to the presence of such a layer as probably of general occurrence. Frioriep ('86, p. 92)



records the presence in the calf of a connective tissue layer dorsad of the chorda. Weiss ('01) found, in embryos of the white rat, an exceptional development of the perichordal sheath in the occipital region, not separated from the horizontal plate of the primitive arch of the atlas (p. 511). The work of Gaupp ('06) on *Echidna*, and Weigner ('12) on man, support the view, first brought forward by Weiss, of a special development of perichordal tissue about the occipital notochord in mammalian embryos. Weiss described the notochord as occupying, in the blastemal stage, a position at the dorsal surface of the segmented portion of the floor of the occipital region; he found that the cranial end of the perichordal sheath grew to assume a globular form; in the chondral stage, cartilage appeared in the perinotochordal sheath, quite independently of the process of chondrification which takes place lateral to and beneath the notochord in the formation of the lateral occipital arches and the basal plate. The more or less spherical cartilage so formed about the notochord is fused, in later stages of development, with the cartilaginous centrum of the atlas, and becomes eventually the extremity of the dens epistrophei. Weiss saw in the cartilage forming the end of the dens epistrophei, which is marked off from the cartilaginous centrum of the atlas by grooves, an element comparable with a vertebral centrum, and concluded that it represented the body of an occipital vertebra, or of a proatlas. Gaupp ('06) found the dens epistrophei in *Echidna* embryos to be composed of the centrum of the atlas and, in addition, of material lying cephalad (and perhaps derived from the basis cranii). This author is of the opinion that the dens epistrophei and the ligamentum apicis dentis represent the anterior reduced end of the vertebral column in which a number of vertebral centra lie imbedded. Weigner ('11) found in a human embryo of 13.5 mm. paired anlagen in the floor of the occipital region, presenting a notch laterally for the hypoglossal nerve, with a deep groove between their caudal extremities occupied by mesenchyma, in which the notochord lies. In the ventral part of this sheet, the hypochordal arch of the occipital region was observed. In the atlantal region, in the tissue dorsad of the notochord, the



paired anlagen of the atlantal centrum were noted. Evidence of the beginning of the occipital centrum was presented in an embryo of 14.5 mm.; the anlage was clearly defined in a later stage (15.3 mm.) as a pair of centers behind the notochord, which pass in a caudal direction gradually into the older centers of the body of the atlas. In man, according to Weigner, the body of the definitive epistropheus with its tooth-process, is developed from three vertebral bodies; those of the occipital vertebra, atlas and epistropheus. The dens itself is formed from the centrum of the occipital vertebra and of the atlas. In the present work no separate centers, in advance of that for the atlantal centrum, were seen, but the tissue about the occipital notochord became condensed next to the chondrifying center of the atlas, and eventually became the cartilaginous apex of the dens epistrophei. In the ossification of the dens epistrophei of cat (as in several mammals) there is, in addition to the bilateral atlantal centers, a single center for the apex of this process (Jayne '98).

Since Froriep's work on the development of the occipital region ('83, '86, '02), the interpretation which that investigator drew from his own results of the relation between cranial and vertebral development and structure has been, in general, sustained; some of the conclusions have, however, been modified by new evidence brought out by recent research. Noordenbos, in 1905, attacked the evidence which has been used in support of the vertebral theory of the skull, claiming in effect that it does not support the homology of parachordal plate and occipital arches with vertebral centra and vertebral arches. Noordenbos rightly objects to the comparison of vertebral bodies, arising as separate, rounded cartilaginous masses, with the parachordal plates, continuous unsegmented masses presenting no trace of special chondral centers. He states that vertebrae arise around the body notochord while the parachordal plate does not. This plate takes origin in one of the three ways mentioned above: as an independent center at the side of the notochord; in connection with the lateral occipital arch; in connection with a hypochordal plate. By the van Wijhe method the vertebral

centra in the mole were found to arise in connection with intra-vertebral capsules, outside the sheath of the notochord. Furthermore, the relation of the neural arch of a vertebra to its centrum differs from that between the lateral occipital arch and parachordal; in the former the parts are primarily independent, in the latter continuous. On account of these discrepancies between vertebral and occipital chondrogenous development, Noordenbos finds weak support for the vertebral theory in the chondrocranium. He says (p. 373): "Ich möchte aus diesen Erscheinungen, im Gegensatz zur allgemein gültigen Auffassung, schliessen, dass für das Chondrocranium die Wirbeltheorie nicht aufrecht zu halten sei." But the vertebral theory, notwithstanding the blows dealt it from the time of Huxley's attack to the present, has shown itself tenacious of life, and the thought uttered by Oken more than one hundred years ago demands deference of the worker of today.

Recent investigations into vertebral development, (Bardeen, Weiss, Weigner) demonstrate the presence of a pair of chondrogenous centers, lying close to the midline and reaching a somewhat higher level dorsally than the notochord, which grow to surround the latter, and so form the cartilaginous vertebral body. There is, in general, apparently a fundamental difference in the original relation to the notochord of the parachordal plate and vertebral centrum. At one spot, only, does the relation of the parachordal to the notochord approach that of a vertebral centrum, namely at the level of the anterior commissure; cartilage is here developed around the notochord. The discovery of processes of chondrification in the perichordal sheath of the occipital region, related eventually to the dens epistrophei and which fulfill the conditions of vertebral central development in general, have been mentioned. It would seem that the evidence presented by these investigators (Weiss, Gaupp, Weigner) justifies the interpretation which has been put forward of rudimentary centra in the occipital region. To this evidence must be added that given by cat. By the interpretation of Weiss of the hypochordal nature of the caudal part of the basal plate, the objection to comparing the lateral occipital arch with the

neural arch of an ordinary vertebra is largely overcome. The lateral occipital arch presents the same relations to a centrum as does the atlantal neural arch, and also the same relations to a hypochordal arch as obtains between the neural and ventral arches of the atlas. That the order of formation of the hypochordal and lateral occipital arches varies somewhat in different species is no obstacle to the interpretation of their equivalency to vertebral structures; the work of Weiss ('01) and Levi ('08) has shown that there is also great variability in the development of the ventral arch of the atlas among mammals.

The fundamental differences between occipital and vertebral development, which have been so clearly indicated by Noordenbos, must, I think, be recognized; but if the interpretation of Weiss be correct, that the occipital develops not as a typical vertebra, but like a specialized vertebra, namely, the atlas, we must admit that there is still evidence of vertebral structure in the skull. This interpretation is in accord with the physiological environment of the region: a transitional zone between a rigid (cranial) and a movable (spinal) division of the axial skeleton. Regarded from this aspect, the structural conditions are seen to change as we pass along the vertebral column toward the head, the form of the vertebrae becoming less typical, due to tendencies along certain definite lines (regression of centrum, development of hypochordal parts). The occipital region shows by its structures the culmination of these tendencies; extremely rudimentary centrum separated from all connection with highly perfected arches, lateral and hypochordal; characters which, to a lesser extent, mark the atlas from a typical vertebra.

There is probably more than a superficial resemblance between the lateral occipital arch and neural atlantal arch in respect to the relations of these parts to nerves. The primary notch formed for the hypoglossal nerve bundles at the side of the parachordal plate, in front of the base of the lateral occipital arch (Noordenbos describes it in the occipital arch), and its subsequent conversion into a foramen, are phenomena which seem to parallel the development of the atlas in the region of the first spinal nerve. In cat embryos of 15 and 23 mm. the first spinal

nerve makes its exit through a notch at the side of the lateral mass of the atlas, between the base of the neural process posteriorly and a short but prominent atlantal process directed dorsad from the lateral mass. The articular regions of the atlas and occipital are comparable, in respect to their relations, to the components of these two skeletal elements. In cat the occipital condyles are formed at the spot where the lateral and hypochordal arches come together, that is on the parachordal plate. This is in agreement with Levi's and Weigner's observation of the relation of condyle and parachordal in man. Likewise, the corresponding articular surfaces of the atlas are formed where ventral arch and neural arch come together, namely at the massa lateralis. The early establishment of similar relations to nerve and articular surface offers a basis for a comparison of parachordal plate and lateral mass of atlas. Apparently these parts are not distinct elements in their relation to the occipital and atlantal arches, but mark a definite locus between the hypochordal arches on the one hand, and neural or lateral occipital arch on the other, constitute the region of articulation, and mark the ventral limit of the exit of the nerves. In this sense the parachordal plate could not include the cartilage ventrad of the notochord; this would fall into the category of the hypochordal arches. The term occipital basal plate would include the originally paired parachordals and the hypochordal cartilage. Comparison of parachordal plate and lateral mass which is here proposed, while attempting to bring out the relations of the occipital and atlas in further detail does not at the same time close the way to a better understanding of a possible relation of atlantal centrum and lateral mass which has been advanced by authors (Hagen, '00).

*Atlas.* Reference has been made to a peculiar character of the atlas, namely the foramen in the neural arch of the adult bone and its relation to the notch in the arch of its cartilaginous predecessor. This atlantal foramen transmits the vertebral artery and the first spinal nerve in cat. It is present normally in most, if not all mammalian orders, holding a position nearer or farther from the cephalic margin of the neural arch, through which varia-



tion of its position the bar of bone forming its cephalic limit is narrow or broad. In man the first spinal nerve and vertebral artery normally traverse a notch in the superior margin of the neural arch; in some instances, however, they pass through a foramen produced by osseous bridging of the notch. Bolk ('99) has pointed out that the usual conditions are indicative of regressive processes in the formation of the human atlas, that in man this bone is reduced in mass as compared with the atlas of those animals in which an atlantal foramen obtains. The bony reduction goes hand in hand with the imperfect development, and probable loss to some extent, of muscle and nerve in the dorsal part of the neck at the level between epistropheus and occiput. The recognition of the human atlas as an atypical example of the form which generally prevails in mammalia is helpful in approaching problems of structure in the head-neck region. Out of the recently much discussed phenomenon of manifestation of an occipital vertebra or assimilation into the occiput of the atlas (Swjetschnikow '06, Kollmann '07, v. Schumacher '07, Smith '09, Glaesmer '10), the question has presented itself to me as to the development of parts in relation to the atlantal foramen. A number of observations on the development and comparative anatomy of the atlas have been made and will be reported in another place. At present I wish to discuss only those which have some bearing upon the occipital region.

The atlas of the smallest cat embryo studied (10 mm.) was represented in cartilage by a pair of neural arches, a small centrum about the notochord, and the beginnings of the hypochordal arch. The expanded base of the neural arch (lateral mass) sends dorsad in front of the latter a small, blunt atlantal process. The notch between the process and arch lodging the first spinal nerve and vertebral artery, is the first step toward the formation of the atlantal foramen. In the stage represented by the model, the notch is relatively deeper, owing to the increase in length of the atlantal process, which is now in the form of a short bar. The base of this bar, anteriorly, participates in the articulation with the occipital condylar surface; its dorsal extremity is free. The boundaries of the foramen are



completed, in the full term fetus, by connective tissue stretching between the free extremity of the anterior bar and the neural arch, anterior to the first spinal nerve.

Observations on the sulcus in the atlantal neural arch for the first spinal nerve in man have been made by Macalister ('93), who gave the name post-glenoid tubercle to the process which rises from the lateral mass and limits the sulcus anteriorly. This process was found to vary considerably in its extent, an observation easily verified in even a small series of specimens. Macalister also noted the presence of an independent bony center in the ligament completing the atlantal foramen in the atlases of young skeletons. The question, whether this represents the typical mode of origin of the bony rod which, in man, completes the atlantal foramen, remains for future enquiry. Separate ossifications in the region of the neural arch of the atlas, between it and occipital, have several times been observed in man. Trolard ('92) found, in two instances, in the posterior ligament of the atlanto-occipital joint an osseous bar placed horizontally, in one case nearly reaching the midplane. The possibility of relation of these independent ossicles in man to the arch of the atlas has, I believe, not been considered. It is well known that an osseous element in the posterior occipito-atlantal ligament, compared with the proatlas of reptiles and extinct amphibia, has been described for *Erinaceus* (see Baur, '94). The possibility of the proatlas being a component of the atlas in a persistent type was many years ago suggested by Osborn ('00). The question of special interest which I wish to mention here in regard to the post-glenoid tubercle, the bar of bone completing the atlantal foramen and the ossicles in the posterior occipito-atlantal ligament, is whether these structures may not possibly represent parts of one element which, in a primitive state, was separate from, but closely related to the atlas.

*Occipito-atlantal articulation.* The discovery by Fischer ('01) of a single, horseshoe-shaped surface at the ventral margin of the foramen magnum in embryos of *Talpa*, articulating with the ventral arch of the atlas would seem to lessen the gap between reptilian monocondylic and mammalian dicondylic articulation.

That the condition in *Talpa* is primitive, and not secondarily acquired, is supported by Gaupp's ('08) observation of a similar atlanto-occipital articulation in *Echidna*. Whereas, in cat, the occipito-atlantal articulation is apparently dicondylic primarily, the joint surfaces are located to a considerable extent upon the basal plate, as well as on the lateral arch, and meet corresponding surfaces of the ventral arch of the atlas and its lateral mass, including the atlantal process. The participation of the basal plate in the articular surface is evidence of greater proximity of the two condylar surfaces toward the median plane than in those mammals (*Lepus*) where the surfaces are restricted to the region lateral to the foramen magnum. This embryonic state of the condyles is, therefore, apparently intermediate between typical dicondylicism and the condition observed by Fischer in *Talpa*. In the later development of cat embryos the condylar surface grows further dorsad by the side of the foramen magnum.

If we accept the comparison of occipital and atlas, we must throw aside the idea of that form of articulation between them, such as exists between the centra of typical vertebrae.

The basal plate in the cat is a derivative of the parachordal cartilages and hypochordal arches and therefore the development of an articular surface upon its caudal margin must involve either one or both of its constituent elements. The point to be emphasized now is the fact that a part of the occipital element, which apparently is as distinct from the centrum as the lateral mass is distinct from the body of the atlas, enters into the constitution of the condyle; that is to say, the condyle belongs to an arch structure.

Further study of that region dorsad of the condyle which is characterized by a notch traversed by veins is necessary before any conclusion can be drawn regarding its significance; it seems not improbable that it may have to do with the foramen condyloideum.

*Plane of the foramen magnum.* Although a marked flexure exists between the head and trunk of cat embryos, this does not explain the basal position of the plane of the foramen magnum

and the occipital condyles referred to on p. 292, which is in contrast with their caudal position in the adult. The immediate cause of this basal position is not difficult to find. Since the lateral occipital arches are quite narrow antero-posteriorly and are joined directly with the otic capsules, it is evident that their basal inclination cannot be attributed either to growth, as in man, or to the presence of wide fissures between them and the ear capsules. The explanation is to be found in the flexures to which the whole chondrocranium is subjected. One result of these flexures upon the longitudinal axis of the cranium is to put its anterior and posterior halves nearly at right angles with each other. It follows that the plane of the foramen magnum stands parallel with that of the floor of the nose. It is of interest to find that, with the formation of the bony cranium, a nearly straight longitudinal axis is substituted for the primary angular axis and, with it, the plane of the foramen magnum becomes less oblique.

A primitive condition, and an exceptional one in the primordial skull of mammals, it seen at the stage represented by the model in the abrupt ascent of the lateral occipital walls from the basal plate. The mammalian chondrocranium, as contrasted with that of lower animals, shows a tendency toward lateral extrusion of these walls; as Gaupp ('06) says: "Die Seitenteile der Occipitalregion sind bei Säugern nicht mehr steil aufgerichtet, sondern nach hinten hin basalwärts niedergelegt am stärksten und vollkommensten beim Menschen."

*Basal fissures.* The fissura basicochlearis posterior of cat is apparently comparable with the opening of the same name in the chondrocranium of *Talpa*, first described and named by Noordenbos ('05). Mead has noted a probable homologue of the posterior basicochlear fissure in *Sus*. In *Lepus* this opening is not present in the stages of development studied by Voit, but an anterior basicochlear fissure was observed by that investigator. These fissures are filled with mesenchyma, which, in the cat embryo, passes into a zone of precartilage at the edges of the opening. The posterior basicochlear fissure, present in *Talpa* embryos of 14 mm., disappears in embryos of 19 to 20

mm. and older, being replaced by cartilage (Noordenbos). Parker ('85) and Fischer ('01) had already shown continuous cartilage in the region between basal plate and cochlear prominence in *Talpa* embryos of later stages. *Tarsius* exhibits still another phase of vacuity at the basicochlear junction, presenting an extensive slit separating the ear capsule and cranial floor (Fischer, '05). In the adult cat, a fissure separates the pars petrosa from the basi-occipital and it appears that in the adult pig the foramen lacerum anterius and the foramen jugulare are connected by a fissure median to the auditory bulla (Mead, '08). The persistence of an original fissure, growing larger as the cranium enlarges, is an interesting phenomenon calling for further study. The posterior basicochlear fissure of *Talpa* is one of several spaces which, as Noordenbos has shown, are derived from the original space separating the independently arising otic capsule from the basal and lateral parts of the chondrocranium. That part of the original space between the base of the skull and the ear capsule is broken up by the later formation of synchondroses, uniting the auditory capsule with the parachordal plate and with the basal region which later enters into the sphenoid; and so there arise a canalis caroticus, an anterior basicochlear fissure and a posterior basicochlear fissure. The observations on cat embryos, presented here, show that the origin of the cartilaginous basal plate and otic capsule are independent and that the fissura basicochlearis posterior is derived from the original space separating the parts.

*Foramen magnum.* In *Lacerta* the dorsal boundary of the foramen magnum is the tectum synoticum. Fischer ('03) found the apparent foramen occipitale magnum of the *Semnopithecus* embryo larger than the future real foramen magnum, the hinder part of the former being closed by membrane. This author remarks on the probability of the membrana atlanto-occipitalis in part undergoing ossification. Bolk ('03) described this region in human embryos, and applies the name incisura occipitalis posterior to a little space filled with membrane made secondarily by the approximation of the dorsal extremities of the occipital side walls in the formation of the foramen magnum. Between



this notch and the tectum posterius is a broad space filled by membrane continuous with that of the incisura posterior. A pair of chondral centers lies in this membrane and a third center stretches through the cranial roof anterior to the tectum posterius. The latter disappears in further development; the pair of centers remaining help to complete the boundary of the incisura posterior, while the membrane between the latter and the tectum posterius undergoes ossification. Voit ('09) also found a space (incisura occipitalis posterior) in *Lepus* between the dorsally open foramen magnum and the tectum posterius, and since, as is explained, the latter belongs to the otic region, the side boundaries of the incisura occipitalis posterior are regarded as the dorsal portions of the occipital pillars. If we compare now the extent of the region between the dorsal confines of the foramen magnum and the tectum posterius in lizard, rabbit, cat, ape and man, it at once appears that there is a progressively increasing area exhibited. This begins with *Lacerta*, where, as Gaupp observed, the tectum is so shaped as to give to the foramen magnum an angle in the dorsal median line, and reaches the great expanse described by Bolk in the human embryo, in which the tectum is far removed from the foramen magnum. It is evident, also, that the term incisura occipitalis posterior has not the same value throughout its application; in the rabbit Voit apparently regards the space limited laterally by the dorsal portions of the occipital pillars as the incisura occipitalis posterior, while Bolk limits the term to only a small part of the region encompassed by the dorsal limits of the occipital walls.

#### *Otic region*

*Position of the otic capsules.* It has been noted (p. 298) that the approximately transverse position of the plane of the otic capsules in the stage represented by the model forms an exception to the general rule in mammals of obliquity of the principal otic axis toward the longitudinal axis of the skull. It will be recalled that the degree of differentiation exhibited by the stage of the model is one, expressed in terms of the skeleton, wherein bone formation has advanced but little; of the mem-



brane bones several have still to make their appearance and none of the purely endochondral ossifications is present. *Talpa* of 27.3 mm., *Semnopithecus* of 53 mm., *Homo* of 8 cm., in which the obliquity of the principal otic axis has been recorded, are all at stages of development in which bone formation is well established and therefore can hardly be compared with cat embryos in the stages under consideration. On the other hand, obliquity of the otic axis is present in the mole of much younger stages, if one can judge by the photographs of Noordembos, and the same is true for the *Sus* cranium described by Mead, which is at approximately the same stage as the *Felis* cranium modeled. Again, Voit states that in *Lepus* the two canalicular parts stand parallel, and only a slight convergence of the cochlear parts is present; the stage is one in which osseous development is advanced (45 mm. gr. L.). Therefore, it appears that the definitive oblique direction of the long axis of the auditory capsule, typical of mammals, is attained at different periods in the species considered, the tendency being toward its early establishment. As in the case of the occipital region, so also with the otic capsule, the position changes with the development of the bony cranium. The shifting of the otic axis from a position at right angles to the cranial base in the chondral stage to one of marked obliquity (cephalo-ventro-mesad) in the osseous stage, can hardly be a result of the straightening of the cranial axis. The factors involved in influencing the change must remain for future inquiry.

Another characteristic of the position of the mammalian otic capsule is its location in the base of the skull rather than in the lateral wall as is the case in increasing degree from this class back to lower vertebrates. In mammals, not only does the cochlear capsule hold a basal position, but the phylogenetically older canalicular part is rotated ventralward as well as caudalward. Toward the attainment of this mammalian peculiarity the cat cranium, in the stages under discussion, presents what seems to be the initial steps. Though the cochlea, a phylogenetically later acquisition to the ear, asserts itself early in claims for space, the relations established in the chondocranium

are readjusted in the bony cranium, the cochlear capsules being widely separated by a broad processus basilaris, and are themselves relatively smaller in the adult skull.

*Origin of the cartilaginous otic capsule.* In discussing the observations which were made on the chondrification of the otic capsule two questions of special interest present themselves: the relation which the origin of the cartilaginous capsule as a whole bears to the rest of the cranium; the original relation between the pars cochlearis and the cartilaginous basis cranii. The first question was raised by Huxley and there was sufficient evidence, notwithstanding the crude methods of his time, for the advancement of the theory of intrinsic skeletal capsules for each of the sense organs, ear, eye, and nose. Subsequent discovery has tended to confirm the truth of this theory, the evidence coming, as might be expected, chiefly from the lower vertebrates. What mammalian crania will show must wait until the study of successive developmental stages of species has been repeated by modern methods. By the van Wijhe method, Noordenbos has presented, recently, very strong evidence of the independent origin of the cartilaginous otic capsule in Talpa; similar results were obtained for rabbit, ox and pig.

The second question has arisen in connection with Gaupp's theory of the reformation of the cranial base to contribute a supporting wall for the cochlear duct. There appears to be in the reptiles a beginning development of the cochlear capsule at the expense of the basal plate. Is the theory supported by evidence from the mammalian chondrocranium and, what concerns us here, does the development of the cochlear capsule in cat throw any light on the problem? So far as mammals are concerned, it is convenient to consider these two questions together. First, it should be borne in mind that only the circumstances of chondrogenous development will be reviewed; the conditions of blastemal structure are here excluded.

In regard to the pars canalicularis the evidence afforded both by sections and van Wijhe preparations indicates that this component of the ear capsule arises independently of other parts of the cranium. There is some difference as to the form of the

cartilage first appearing in the pars canalicularis from what has been observed in other animals. I refer to the observation of a plate of cartilage upon the lateral side of the semicircular canals; in the cat, cartilage forms upon the lateral surfaces of the canals in more or less separate stretches for each canal. The canals are subsequently completely walled and the intervals between them filled through the development of cartilage, but whether this is by extension of cartilage formation from the canalicular walls already established or from independent chondrifying centers was not observed. The more or less compact mass constituting the pars canalicularis is secondarily connected with the lateral occipital arch and parietal plate, although its dorsal margin remains free in the fissura jugulare spurium, and the posterior margin is clearly indicated, even at the 23.1 mm. stage, in the stretch of young cartilage between it and the lateral occipital arch directed toward the jugular foramen. The pars canalicularis is also apparently formed independently of the suprafacial commissure, if one may judge this by the difference in degree of development of these two closely associated parts.

In the pars cochlearis, cartilage was first observed in the region next the pars canalicularis and in the anterior and posterior poles, i.e. in the neighborhood of the suprafacial and basivestibular commissures. There was no actual separation of these chondrifying tracts from one another, no independent centers of cartilage formation. At the stage when the medial wall of the cochlear capsule is in precartilage, the capsule is separated from the chondrified basal plate by a fissure filled with mesenchyma. Union of the capsule by cartilage with the basal plate behind the carotid foramen, with the alicochlear commissure and basivestibular commissure, is brought about secondarily. Between the commissura suprafacialis and anterior pole of the cochlear capsule a smaller degree of difference in development obtains than is the case between this commissure and the pars canalicularis; the ventral end of the commissure, however, appears to be blended, if not actually continuous, with the cochlear capsule. In this discussion, the fact of the continuity of these two parts is important because of the possibility of pa-

rietal if not actual basal relationship and significance of the commissure. The evidence of the parietal nature of the suprafacial commissure may be considered at once, since upon its interpretation depends to a large extent the question of the cochlear relations to the basal plate.

The relations of the suprafacial commissure in cat appear to differ somewhat from those described in other mammals. In *Talpa*, according to Fischer ('01), the roof of the facial canal is made by a thin lamella of cartilage stretching from the pars canaliculus semicircularium to the highest elevation of the cochlear capsule. Fischer regards the walls of the facial canal as made entirely by the ear capsule, and contrasts this condition with the formation of the canal in *Lacerta*, in which, as Gaupp ('00) has found, the foramen for the seventh cerebral nerve lies in the boundary zone between the basal plate and ear capsule. Noordenbos ('05) named the roof of the facial canal the tectum nervi fascialis, and found it connected with the medial wall of the anterior ampullary swelling of the pars canalicularis. Voit ('09) found, in the rabbit, that the suprafacial commissure stretched from the anterior end of the pars posterior of the otic capsule, beneath the prominentia utriculo-ampullaris posterior and right above the superior acoustic foramen, to the roof of the anterior part of the pars cochlearis. In comparing the facial foramen in rabbit and lizard, Voit evidently agrees with Gaupp that, in mammals, the walls of the foramen are in part contributed to by the cochlear capsule. In the dog, the suprafacial commissure bridges over the facial foramen, from the borders of the pars utriculo-canalicularis and the pars sacculo-cochlearis, according to Olmstead ('11). De Burlet ('14) described the commissura praefacialis of *Balaenoptera rostrata* as a cartilaginous bridge between the pars cochlearis and pars canalicularis. In De Burlet's plates VI and VII, however, there is shown what appears to be a connection between the praefacial commissure and lamina parietalis. The relations of the lateral end of the suprafacial commissure in the cat differ from those in the mammals mentioned, with the exception possibly of *Balaenoptera*. Connection between the commissure



and pars canalicularis is likewise seen in cat, but the relation is apparently not the primary one. The primary lateral termination of the suprafacial commissure in the cat is not in the otic capsule, but in the commissura orbito-parietalis, where the latter meets the prominentia ampullaris superior. Continuity with the orbito-parietal commissure (a part of the lateral cranial wall) and, at the same time, partial separation from the otic capsule, is to be regarded as evidence pointing toward the parietal nature of the suprafacial commissure. While I find no reference to a relation between the suprafacial commissure and cranial side wall in descriptions of mammalian chondrocrania, the constant proximity of these parts strongly suggests that the continuity seen in cat may obtain in other mammals. De Burlet's figures of the *Balaenoptera* chondrocranium are of great interest in this connection. Further support of the view of the parietal nature of the suprafacial commissure is offered by the relation of this structure to the exit of the trigeminus. As stated in the description of the orbito-temporal region, the roots of this nerve and the semilunar ganglion lie between the suprafacial commissure and a thickened band of the transverse septum in which is developed an accessory cartilaginous rod resting upon the dorso-medial surface of the ganglion. A similar structure was observed in the rabbit by Voit who interpreted the 'Restknorpel b' as possibly representing the pila prootica of *Lacerta*, a structure of the primary cranial wall. In *Lacerta* the trigeminus makes its exit by way of the prootic fenestra whose boundaries are the prootic pillar and prefacial commissure. In the cat the fifth nerve leaves the cranial cavity between the accessory cartilaginous rod and the suprafacial commissure. Continuity of the anterior part of the otic capsule with a portion of the lateral wall of the cranium in the cat should make us hesitate in assigning entirely otic boundaries for the facial canal. The evidence so far indicates possibly that the foramen faciale in the cat stands in the boundary zone between the pars cochlearis of the otic capsule and the cranial wall, represented by the suprafacial commissure. Such a relation is in harmony with that which obtains in reptiles, to the



extent that the foramen for the facial nerve stands between a sense capsule (otic) and cranial wall (basal plate).

In accordance with our observations, the cochlear capsule in the cat is developed in connection with the pars canalicularis and with the suprafacial commissure which gives evidence of being, in part at least, a parietal structure. The cochlear capsule chondrifies independently of the basal plate, with which it is secondarily united by commissures. Its precocious growth and great bulk encroach upon the broad region occupied in lower vertebrates by the basal plate. If we can, on the evidence given, interpret the suprafacial commissure as a parietal structure in the cat, it would appear that its relation to the cochlear capsule (continuity) affords support to the theory (Gaupp) of the latter having preempted the territory of the basal plate and developed at its expense.

*Foramina acustica and meatus acusticus internus.* The acoustic foramen or fissure and the entrance to the facial canal are established early in the development of the otic capsule. These openings lie between the suprafacial commissure behind and, in the 23.1 mm. stage, are found at the bottom of a shallow internal acoustic meatus. The single acoustic fissure is constricted in its middle so as partly to separate two wide divisions accommodating the vestibular and cochlear nerves. Completely separated foramina were not observed in any of the stages studied. Comparable with this form of acoustic nerve entrance is the single dumb-bell shaped foramen which Mead has described in *Sus*. In the rabbit (Voit) and the dog (Olmstead) there are separate foramina for the vestibular and cochlear nerves. In the cranium of *Talpa* of the stage described by Fischer there are also two separate acoustic foramina; but in younger embryos Noordenbos found a round *porus acusticus internus*, in the bottom of which openings for the cochlear and vestibular nerves were not clearly separated on account of lack of chondrification. The persistence of a single opening in the cat speaks for tardy development of the medial wall of the ear capsule, a tendency characteristic of the lower vertebrates. The relative positions of the foramina, one to the other, or of the nerves, where a single

opening exists, while in general constant for mammals, shows an interesting variation in the cat. In mammals these openings lie approximately one above the other, so that a superior and an inferior acoustic foramen, for the vestibular and cochlear nerves respectively can be spoken of. In the cat, at the stage of 23.1 mm. the vestibular nerve occupies the antero-lateral part of the fissure; the cochlear nerve the medio-caudal end. The antero-posterior order of the acoustic rami recalls the condition in reptiles. Although a common meatus for the two divisions of the acoustic nerve occurs in birds (Tonkoff, '00) this is separate from the exit of the facial nerve, and therefore is not comparable with the internal acoustic meatus of mammals, which includes separate passages for the vestibular and cochlear rami and for the nervus facialis. The development of the internal acoustic meatus begins very early and seems to be a result, not of a depression of the medial otic wall, but, as Voit remarks, of the elevation of the surrounding cartilaginous parts. In the cat, the elevations are made by the suprafacial commissure anteriorly, the otic extremity of the basivestibular commissure posteriorly and the prominentia utricularis dorsally and laterally. These three parts rise above the level of the cochlear roof and form three sides of the meatus acusticus internus and porus acusticus. The low, medial and ventral side is the roof of the cochlea. Of these three elevations, that of the suprafacial commissure is most prominent and is probably the chief factor in determining the presence of the meatus.

*Foramen perilymphaticum.* Since Fischer's ('03) description of the derivation of the aquaeductus cochleae and fenestra cochleae from the perilymphatic foramen, several observations have been made by other investigators concerning this interesting phenomenon. Fischer found in an embryo *Semnopithecus* that the downward directed opening at the basal side of the ear capsule was divided into two parts by a process which sprang from the anterior margin of the opening and extended backward. The larger lateral part looked out from the free outer surface of the skull and was closed by thick membrane; it was identified as the fenestra cochleae. The medial smaller opening, on a

higher plane, looked inward, was traversed by the perilymphatic duct and was recognized as the aquaeductus cochleae. Following his observation, Fischer states that Hertwig's enlarged model of the otic region of man shows obscurely the process in question. I find in this model a free corner of the cochlear wall projecting forward from the posterior margin of the large foramen so as to divide the opening into a large lateral and a very small medial part. In the rabbit, Voit found the conditions exactly as Fischer saw them in *Semnopithecus* and named the dividing process the *processus intraperilymphaticus*. Macklin ('14) described the intraperilymphatic process in the human embryo as a "short conical projection directed forward from the inferior utriculoampullary prominence." In the cat, a process of the caudal wall of the cochlear capsule, adjacent to the basivestibular commissure, projects laterally, and tends to separate the perilymphatic foramen into two parts. We find, therefore, that the perilymphatic process in the ape and the rabbit is directed backward, and in man forward, while in the cat the process which separates the perilymphatic foramen into intra- and extracranial openings is directed laterally. If these be comparable processes they probably indicate, merely, differences in the place where chondrification begins in the septum dividing the perilymphatic foramen.

*Cavum vestibulare.* It is apparent from the foregoing description of the vestibular cavity that the conditions present in the embryo of 23.1 mm. are far from what obtains in the adult mammalian temporal bone. Only on the most general lines are the form and relations seen in the bony walled vestibular spaces of adult cat referable to the conditions of the cartilaginous otic capsule. Such characteristics of adult structure as the sharp delimitation of special recesses for the utricle and saccule, apparently are not even indicated in embryos considerably further developed than those of the stage modeled. Between these early stages and adult conditions many processes of formation are involved, about which almost nothing is known.

If the vestibular cavities of the cat embryo of the stages described present but few indications of their adult form, they

do show a certain agreement with conditions observed in the lower groups. The tendency to subdivision of the vestibule into anterior and posterior rooms is strongly suggestive of the conformation of the *cavum vestibulare* in *Lacerta*, as described by Gaupp. The contents of the anterior and posterior vestibular cavities in the cat appear to be comparable with the parts contained in the *cavum vestibulare anterius* and *cavum vestibulare posterius* of lizard. The anterior and posterior acoustic foramina in lizard and cat are similarly related to the vestibular cavities. Furthermore the relative positions of the two spaces, one to the other, and to the otic capsule as a whole, are strikingly similar in the two forms. Such a comparison must accept of necessity the homology of the boundary structure between the vestibular cavities. In the cat this is the medialward projecting ventral part of the *massa angularis*, the part designated *crista intervestibularis*. In the lizard it is the *septum intervestibulare*. Gaupp describes the latter as a transverse vertical partition between the two vestibular cavities, presenting a lateral opening filled with membrane and a medial foramen for the *utriculus*. The *crista intervestibularis* is likewise transverse in position, but it cannot be called a partition since it fails to extend entirely across, between the anterior and posterior cavities. While it possesses no opening, yet, by its failure to reach the medial vestibular wall, a space is left between the latter and the free medial edge of the *crista*, by which the anterior and posterior cavities are put into communication and which is occupied by the *utriculus*.

*Lamina parietalis and tectum posterius.* Decker ('83) found the parietal plates in cat embryos of 5.5 to 6.1 cm. bent inward, which is not the case in the younger embryos of the present study; rather, the dorsal, irregular margins are turned a little laterally. Decker's observation is interesting in proving that the parietal plate participates in the cranial roof, to only a slight extent, as his figure shows, but, nevertheless, marking an unusual development of this part of the cranial wall for mammals. The *lamina supracapsularis* of *Echidna*, remarkable for its great breadth and its continuity with the ear capsule, is, in its anterior part, com-



parable with the lamina parietalis of placental mammals, also to the hinder portion of the taenia marginalis of *Lacerta* (Gaupp, '08, b).

Chondrification of the parietal plate independently of the ear capsule was not observed in the series of *Echidna* studied by Gaupp, but it was noted that the plate was separated from the occipital pillar caudally and from the ala orbitalis rostrally by stretches of unchondrified tissue. Noordenbos is apparently the first to have observed the independent origin of the parietal plate and its secondary union with other parts of the skull. In *Talpa* of 11 mm. this cartilage was dorsad of the ear capsule and immediately in front of the tectum interoccipitale; its union with the otic capsule occurred very soon after its appearance. Evidently the parietal plate in *Talpa* does not unite posteriorly with the occipital arch, but with the tectum interoccipitale. The latter appears in the mole at the same stage as does the parietal plate, in the form of an independent piece arched over the cerebellar region. Noordenbos observed its union, first with the occipital arch, then considerably later with the parietal plate. On account of the primary union with the occipital arch Noordenbos is inclined to adopt the term 'tectum interoccipitale' as more correctly expressing its relations than the terms 'tectum synoticum' and 'tectum posterius.' Whether the tectum arises by paired anlagen in the mole could not be stated; its origin in the rabbit was observed by Noordenbos to be paired.

In regard to the origin of the parietal plate in cat, I am unable to present a conclusion, owing to lack of material at a critical stage in the development of this part. Apparently primary conditions similar to those described for *Talpa* obtain in *Felis*. A cartilage, which I have called lamina parietalis, arises by paired beginnings in the form of triangular plates, above the interval between the occipital arch and pars canalicularis. At a later stage the parietal plate has united with the lateral occipital arch and presents a prominent angle toward the mid-dorsal line, which is the beginning of the tectum posterius. At this stage (15 mm.) there is a faintly stained tract of cartilage in the region of the future commissura orbito-parietalis, but extending caudad into



the otic region. There remains, however, a wide gap, equal in extent to the dorsal margin of the ear capsule, between this tract of cartilage, which I have referred to as the beginning of the commissura orbitoparietalis, and the parietal plate. Now in the mole, Noordenbos found a cartilaginous plate immediately anterior to the tectum interoccipitale and identified it as the parietal plate. It united subsequently with the otic capsule, opposite the junction of the anterior and middle thirds of the anterior semicircular canal (synchondrosis parieto-canalicularis), with the anterior margin and ventral end of the tectum interoccipitale, and also with the ala orbitalis. The last connection came about through the synchondrosis orbito-parietalis, by extension of the lateral hinder angle of the ala orbitalis.

In sections of cat embryos of 17 mm. a parietal plate of young cartilage extends from the occipital arch to the commissura orbito-parietalis. The arch of the tectum posterius is represented merely by a prong, springing on either side from the broad plate of cartilage forming the caudal end of the parietal plate, and which is united by stretches of young cartilage with the pars canalicularis and lateral occipital arch. The anterior end of the parietal plate, while approaching the otic capsule at the level of the middle of the anterior semicircular canal, is still not united with it, but is separated by a plane of mesenchyma. In later stages (24 to 30 mm.) van Wijhe preparations show that chondrification is less advanced, in the narrow part of the parietal plate, where the inconstant union with the pars canalicularis occurs (p. 310), and where union has occurred with the occipital arch and otic capsule. There seems to be no doubt of the cartilaginous plates, identified as the beginnings of the parietal plate, going to form also the tectum posterius (the arch is complete in the 23.1 mm. stage) and also the posterior part of the parietal plate. The relation of the commissura orbito-parietalis to the otic capsule anteriorly (the so-called parieto-capsular commissure) is evidence of the probable homology of this cartilage with the parietal plate of Talpa, as described by Noordenbos. There is no evidence of another center of chondrification between the termination of the orbito-parietal commissure and the tectum pos-

terius, and it is doubtful, notwithstanding the great space between these parts, if another center does develop and enter into the formation of the parietal plate. In favor of this assumption is the brief period between the stage when the orbito-parietal commissure and tectum posterius are separate (15 mm.) and the stage when they are joined (17 mm.). Apparently, in the cat, the parietal plate is formed by the coalescence of two cartilages arising independently, one, mostly anterior to the otic region, which gives rise also to the larger part of the orbito-parietal commissure and to the parieto-capsular commissure; the other dorsal of the interval between the lateral occipital arch and pars canalicularis which unites with these parts, forms the broad caudal portion of the parietal plate and also gives rise to the tectum posterius.

*Facial and acoustic nerves.* The suprafacial commissure, forming the roof of the primary facial canal, separates the facial nerve from the ganglion semilunare. As the seventh nerve (including the pars intermedia Wrisbergii) leaves the canal, the geniculate ganglion is formed on its dorsal side. This is in contact with the ganglion of the trigeminus, both structures lying outside the plane of the fenestra sphenoparietalis. The lateral opening of this primary facial canal should be compared with the foramen faciale of reptiles. A foramen or canal, traversed by the facial nerve beyond the ganglion geniculi, is a new acquisition for mammals and not to be found in the reptilian cranium. Such is the stretch which Fischer ('01) has described in *Talpa*, roofed over by the "ganz dünne Knorpelspange" (p. 504), separating the proper facial opening from the hiatus spurius. Also the foramen faciale externum of the tegmen tympani of the rabbit forms an acquisition to the primary facial canal, whose lateral opening in the tympanum is the apertura tympanica. This conception of the facial canal is partly in accord with that of Vrolik ('73). In the cat, at the stage modeled, the exit of the primary facial canal is at the level of the ganglion geniculi (position of the future bony hiatus canalis facialis) and outside the cavity of the chondrocranium. In the bony cranium of cat, and probably in later stages of the chondrocranium, the exit from the cranial cavity is by the apertura tympanica.

Regarding the course of the seventh nerve in the sulcus facialis, it is to be remembered that in adult *Felis domestica* the second part of the facial nerve traverses an open groove in the medial tympanic wall (the rule in mammals), whose lateral boundary is the ossified processus paroticus (crista parotica of van Kampen, '04). Neither in the embryo nor in the adult does the free margin of the parotic crest incline toward the vestibular wall in the formation of a canal. In *Felis pardus*, however, the nerve does run in a closed canal (Denker, '99). The groove in the adult domestic cat begins anteriorly at the apertura tympanica and terminates posteriorly opposite the level of the tympanohyale.

Finally, reference should be made to the discovery by Spence ('90) in the adult and new born cat of a bony or cartilaginous support of the chorda tympani, projecting from the tympanic bone. Bondy ('07) has confirmed this observation, finding the process not only in cat but in a number of other mammals. No evidence of the support was found in the stages described here; its formation takes place according to Bondy, late in fetal life.

*Acoustic nerve.* Retzius ('84) described the nervus acusticus of the cat as dividing into two or three chief branches, preferring two in his account. I found this mode of branching in the cat embryo of 23.1 mm., and it may be remarked that, in our present state of knowledge of the distribution of the acoustic nerve in the cat, the nomenclature of Retzius seems preferable to one which attempts to represent the origins of the nerve fibers. For, in the case of the ramus posterior, cells of the vestibular and spiral ganglia are intimately associated, and no safe conclusion on the origin of fibers of this ramus can be reached without further neurological investigation. The small twig from the anterior ramus to the sacculus appears to correspond with the ramulus maculae sacculi pars superior found by Voit ('07) in *Lepus*.

#### *Orbito-temporal region*

*Hypophyseal cartilage.* Chondrification of the base of the cranium in the hypophyseal region has been observed in several mammals to take place independently of the rest of the chondro-

cranium. Parker ('75) observed "a secondary growth of cartilage beneath the pituitary body" in *Sus*. A sphenoidal cartilage, independent of the occipital skeleton of the ox, is described by Froriep ('86) as lying beneath the hypophysis. This observation is confirmed by Noordénbos ('05) who found, moreover, that the cartilage was paired. Noordénbos has discovered, by van Wijhe's method, the origin of the middle piece of the cranial base by the fusion of several small islands of cartilage in the crania of mole, rabbit and pig. For these pieces, which surround the stalk of the hypophysis, Noordénbos has proposed the name 'insulae polares.' Wincza ('96) noted the independence of the cartilaginous basi-sphenoid and alisphenoid (ala temporalis) in cat. As we have seen, the cranial floor, beneath the hypophysis, is first represented by a crescentic cartilage, which soon grows around the stalk of the hypophysis, probably completely surrounding it, although this was not actually observed. The formation of the sella turcica is brought about by the union of the hypophyseal cartilage anteriorly with the trabecular plate and posteriorly with the basal plate, the former contributing the tuberculum sellae, the latter the crista transversa which is the beginning of the dorsum sellae.

*Fenestra basi-cranialis posterior.* The existence of an opening in the basis cranii between the anterior end of the basal plate and the hypophyseal cartilage is merely temporary in the cat. In embryos of 12 mm. the fenestra basi-cranialis posterior has no lateral limits, since the cochlear capsule is not yet joined with the basal plate. The anterior boundary is made by the hypophyseal cartilage, so that the fenestra lies, not within the basal (parachordal) plate, but anterior to it, as Noordénbos insists.

*Crista transversa and dorsum sellae.* The crista transversa represents the anterior, dorsally turned, free edge of the basal plate. The upward bend of this margin just prior to the fusion of the basal plate and hypophyseal cartilage, can be seen in sagittal sections of embryos of 12 mm. and its identity with the crista transversa proved by sections of 15 mm. specimens in which the line of fusion of the two plates is still distinct. Noordénbos found that the dorsum sellae of *Talpa* is formed from the anterior mar-



gin of the parachordal plate. In the cat, the dorsum sellae is also in part at least, derived from it, but probably the greater part is derived from the mesenchyma above the crista transversa and in front of the end of the notochord, in which the small cartilaginous nodule was found in the smaller embryos.

Let us consider briefly some observations regarding the dorsum sellae. I believe no comparative study of its formation has been made. This structure reaches a high development in man and apes. Fischer ('03) found in a *Macacus* embryo the upper border isolated from the rest of the dorsum sellae, lying as a transverse bar, and terminating laterally in the posterior clinoid processes. This author also points out that in the adult human cranium a groove or ridge stands between the dorsal border and the clivus and puts forth the suggestion that this structure, isolated in *Macacus*, is probably genetically foreign to the basal plate. Voit ('09) supports this view by the discovery in *Lepus* of a partial separation of the dorsal part of the saddle-back by a wide foramen from the rest of that structure. Fawcett ('10) found in human embryos of 19 and 21 mm. a rounded mass of cartilage, behind the pituitary body, connected with the clivus region by a fibrous bridge; he concluded that the dorsum sellae arises independently in man. In his study of the primordial cranium of *Talpa*, Fischer ('01) found the hypophyseal fessa to be a slight depression and noted the absence of a dorsum ephippii. Noordenbos ('05) speaks of a weakly developed dorsum sellae turcicae in mole embryos and states that it is a structure of the parachordal plate. This investigator observed that in embryos of 11 mm. the caudal end of the polar plate, lay somewhat beneath the cranial end of the parachordal plate. With the union of these parts the hypophyseal fossa is formed, limited posteriorly by the projecting anterior end of the parachordal plate.

Fischer's and Voit's observations point to the origin of the upper part of the dorsum sellae as possibly distinct from the basal plate in *Macacus* and *Lepus*, and, according to Fawcett, the origin of the dorsum sellae in man is from an independent center of chondrification. Noordenbos found the dorsum sellae of *Talpa* as a product of the parachordal (basal) plate. These observa-



tions suggest the possibility of the dorsum sellae of different species not being strictly homologous. Gaupp says ('00, p. 538) "Dass die hinten begrenzende Crista sellaris, wie sie bei dem Chondrocranium der Saurier (und auch bei dem der Vögel) vorkommt, der Sattelsehne des Säugercranium entspricht, ist allgemein anerkannt." But does the crista sellaris of reptiles compare with the dorsum sellae of mammals? And are the latter strictly homologous in the different orders of mammals?

This is not the time for a full discussion of these questions which requires a larger basis of observations, but a few comments may be offered. Regarding the homology of the dorsum sellae among the mammals the following may be noted. The saddle-back of the chondrocranium of *Echidna* (Gaupp '08), *Talpa* (Noordenbos, '05), *Caluromys* and *Didelphis* (my own observation) is very low. As already stated, Fischer denies its presence altogether in the *Talpa* embryo he studied. In contrast with the insignificant low ridge-like dorsum ehippii of these species is the high saddle-back of the chondrocrania of man, apes, rabbit and cat. In all these species evidence is at hand indicating the presence of an element in the dorsal part of the saddle-back, more or less independent of the base of that structure. In embryos of cat smaller than the stage modeled, the notochord terminates in a mass of mesenchyma which surmounts the crista transversa, whereas in the latter stage it ends in the perichondrium of a cartilaginous tubercle which rises from the middle of the transverse crest. This tubercle is developed from the mesenchymal mass, and may possibly have its beginning in the prochondral nucleus observed in the embryos of earlier stages (p. 327). In the cat embryo of the stage modeled, the dorsum sellae is formed to some extent also from the up-turned edge of the parachordal plate (crista transversa). It would seem, in respect to the contrast between the caudal limits of the hypophyseal fossa in the two groups here presented, that there is an element present in the one which is not found in the other, or that in one group a simple crista transversa forms the back of the pituitary fossa, whereas in the other a crista transversa plus an additional element enters into the construction of a dorsum sellae.

There is nothing in the model of the cat embryo to indicate the presence of post-clinoid processes, which are found in the bony cranium and are therefore formed later. These processes in connection with the processus interclinoideae, Voit regards as vestiges of the primitive cranial wall in this region. Interclinoid processes have often been observed in the adult cranium of man, and Fischer has noted them in the chondrocranium of *Macacus*.

*Foramen hypophyseos.* The foramen hypophyseos is converted into a canal by the growth in thickness of the floor of the sella turcica. In embryos of 17 mm. the stalk of Rathke's pouch is still intact as it lies in the canal. In the floor of the sella turcica of the stage modeled, the hypophyseal canal is present, but only vestiges of the stalk are apparent in it. Arai ('07) has described a bony walled canalis cranio-pharyngeus in the cat, containing a vein and a hypophysis accessoria cranio-pharyngei; also an epithelial-lined blind canal interpreted as a possible vestige of the stalk of Rathke's pouch. Voit has criticised Arai's homology of the cranio-pharyngeal canal and the hypophyseal foramen in the rabbit, asserting that the former is a secondary development, occurring in a position caudad of the location of the hypophyseal foramen. This criticism is not pertinent in the cat; the foramen and the canal have the same location.

*Ala temporalis.* Wineza ('96) described the boundary line between the alisphenoid and basisphenoid (properly lingula) in embryos of the cat. My observations are in accord with this description; a zone of perichondral tissue standing between a chondrified processus alaris of the basis cranii and a broad cartilaginous plate, ala temporalis, in relation to the Gasserian ganglion. The complete independence of the alisphenoid in the cat led Wineza to investigate its relation to the cranium in other mammals with the following results: In the dog embryo, a separating zone was found between the cartilaginous alisphenoid and basisphenoid; in embryos of the polar bear complete separation of the two parts, with a small wedge-shaped cartilage in the cleft; in man, a joint between alisphenoid and basisphenoid, recalling the relation between the head of the femur and the acetabulum; in hedgehog embryos a boundary between the basi-

and alisphenoid behind, both parts united in front in three of the stages studied. Wineza's observations have since been confirmed for man by Levi ('00), Gaupp ('02) and Fawcett ('10); for the dog by Olmstead ('11). Noordenbos ('05), Voit ('09) and Fuchs ('10) have seen the separating zone in *Lepus*. Noordenbos also found the ala temporalis a free process in the pig and horse. A characteristic of the synchondrosis between alisphenoid and basisphenoid of the cat described by Wineza, is its oblique course from behind, forward and medialward. Whether the obliquity is a constant feature of the separating zone of the ala temporalis in other forms studied by Wineza is not specifically stated. Figure 8 of Wineza's paper shows a cleft separating the two parts in the polar bear, having the same direction as the boundary zone in the cat. Macklin ('14) found in a 40 mm. human embryo that the connection between the lateral portion of the ala and the processus alaris took place between the ventral surface of the latter and the subjacent ala.

In contrast to the type of independent ala temporalis there has been observed another type, characterized by its continuity with the basis cranii. Wineza saw no trace of a boundary between basisphenoid and alisphenoid in the chondrocranium of embryos of the horse, pig, sheep and calf. Noordenbos, however, as just stated, disagrees with the observation on the horse and pig; he finds the ala temporalis in mole to be a process of the side of the sella turcica. In *Echidna*, Gaupp identified the ala temporalis in the small continuous process which springs from the side of the sella turcica, laterad of the carotid foramen.

From these records we learn that, in a variety of mammals, the ala temporalis is more or less distinct from the rest of the cranium, being separated from a basal process by a stratum of some tissue other than cartilage, or even by a cleft of greater or less extent; whereas in others the ala is a simple process continuous with the cranial base. Furthermore, Noordenbos, from his own observations, recognizes two types of embryonic origin of the ala temporalis in mammals; one type, represented by the mole, in which the ala arises as a lateral process of the pole-plate; the other type, represented by the rabbit, wherein the ala arises

independently and unites secondarily with a process (processus alaris) of the margin of the sella turcica.

Of the many questions yet unanswered regarding the significance and relations of the ala temporalis, one only will be discussed here. The cat belongs to the type in which the temporal wing is more or less separate from the rest of the cranium, and in which the connection is an indirect one through a processus alaris. The successive stages of development of the ala temporalis in the cat seem to throw some light on the nature of the differences between the temporal wing of the continuous type and that of the separate type. The following discussion deals with this question.

We may first compare the development of the ala temporalis in the two types, i.e., the one in which the temporal wing is continuous with the rest of the cranium and that in which it is more or less separate. For the first, *Talpa* may be chosen as an example; for the second, the cat. The following results have been brought out by Noordenbos in his study of the mole. In embryos of 12–13 mm. three lateral processes at the side of the pole-plate (basisphenoid) are described. With the posterior one of these processes (basicochlear synchondrosis) we are not here concerned. The intermediate process, knee-formed, extends from the pole-plate in front of, and then bends laterally around, the carotid artery passing backward to join the cochlear capsule. The anterior process ends free. In embryos of 14 to 17 mm. the latter has grown so as to come into relation with the Gasserian ganglion. In still older embryos (17–19 mm.) the anterior limb and knee of the middle process grow out in connection with the anterior process to reach the under surface of the semilunar ganglion and thus is formed the ala temporalis. "This little plate is connected with the lateral margin of the basisphenoid through the processus anterior and the anterior limb of the processus intermedius." Noordenbos states that the largest part of the ala temporalis is contributed by the anterior lateral process of the pole-plate; a small part arises from the knee of the middle lateral process. Union of the two elements of the ala occurs in embryos of 20 to 25 mm., but there remains for a long time, a small



fissure-like opening in the root of the ala as the last vestige of the original gap between the anterior and middle lateral processes of the pole-plate. The middle process, the synchondrosis sphenocochlearis lateralis, is, in its caudal half, the part named by Fischer in a later stage the 'trabecula alacochlearis.' Noordenbos has shown, however, that the trabecula alacochlearis genetically has its anterior connection, not with the ala, but with the basisphenoid, and only secondarily unites with the hinder margin of the root of the ala temporalis.

Let us consider now, the development of the ala temporalis in the cat and compare it with that of the mole, taking as a point of departure the stage of 17 mm. In the cat there arises from the side of the sella turcica, a precartilaginous bar, which bends about the carotid artery, its anterior limb (processus alaris) crossing transversely in front of the vessel, its posterior limb (commissura alicochlearis) extending backward upon the side of the artery and joining the cochlear capsule. Here, then, are elements present in both mole and cat which are in agreement in several important relations. In order further to identify this combined alar process and alicochlear commissure of cat with the middle process of mole, it should be recalled that it is derived from the commissural element of the earliest stage (12 mm.) the relations of which to the base of the cranium, to the cochlear capsule and to the carotid artery are equivalent to the relations of the middle process of mole at its first appearance (12 to 13 mm.). Comparing, next, the relations of the middle process of mole and the precartilaginous derivative of the commissural element in cat, with parts lying laterally, there is present in each case an element, separate from the process under consideration, related to the semilunar ganglion. In *Talpa* this element, derived from the anterior process, grows out alongside the anterior limb of the middle process, from which, however, it is at first separate. It is noteworthy that the former extends somewhat caudally as well as laterally, and, in conformity therewith, the space intervening between it and the middle process extends from behind, forward and medialward. In embryos of 20 to 25 mm. these two pieces of the ala temporalis unite, the anterior limb of the



middle process being thereby taken into the root of the ala. In cat embryos of 17 mm., the ala temporalis, the derivative of the mesenchymal alar element in relation to the Gasserian ganglion, stands lateral to and in front of the anterior limb of the commissural element from which, however, it is separated by a layer of mesenchyma (p. 329). Caudally the ala temporalis is separated from the posterior limb of the commissural element by the space which later results in the incisura lacera. The lamina presents then an oblique line of junction with the anterior limb of the commissural element, extending from behind, forward and medially. Later stages (23.1 mm.) as already described, show along this line a persistent sign of the separation of ala temporalis and the arch made by the processus alaris and aliochlear commissure, in the perichondral boundary zone first described by Wincza. The anterior process, which in mole forms the greater part of the ala temporalis, is entirely separated by an interval from the anterior limb and knee of the middle process for a time; the alar element, which in the cat forms the entire ala temporalis, is separated from the commissural element by an interval (incisura lacera) only in its caudal part. It will be recalled that Wincza found in the polar-bear the alisphenoid separated from the basi-sphenoid by a cleft which, as the figure shows, runs from behind forward and medialward. Also the same author found in the hedgehog a limiting zone in the posterior part only of the ala temporalis.

Apparently the alar element of the cat and the anterior lateral process of the pole-plate of the mole are, in several respects, comparable. The one real difference between the alar element of the cat and the anterior process of the mole lies in the relation of these parts to the side of the sella turcica: in the mole the element in question is connected medially with the sella; in the cat it passes medially into the commissural element, without independent connection, if any at all, with the side of the sella turcica. There is a reduction, if not entire absence, of a medial part of the alar element, next to the hypophyseal cartilage comparable with the root of the anterior process of mole.

The differences between the independent ala temporalis of the cat and the continuous ala of the mole are apparent rather than real. A very simple explanation removes these apparent differences. 1) The alar element of the cat is wanting in a medial part, comparable with the origin of the anterior process from the pole-plate in the mole. Excepting in the undifferentiated mesenchymal continuity of the alar element and anterior limb of the commissural element, there is no indication of a connection between it or its derivative, the ala temporalis, and the side of the sella turcica. 2) The processus alaris is compound in the mole, being derived from the root of the anterior process plus the anterior limb of the middle process; it is simple in the cat, being composed of the anterior limb of the commissural element only. The processus alaris of the cat is therefore comparable with only the caudal part of the ala temporalis of the mole, i.e., with that part derived from the anterior limb of the middle process. 3) The alar element of the cat and its derivative fails to unite with the commissural element and its derivatives, and there remains throughout the cartilaginous stage of the ala temporalis, a synchondrosis representing the limiting margins of the two elements. In the mole, partial fusion takes place between the comparable elements, but a cleft-like vestige remains locating the original separating space. Perhaps the conditions in the hedge-hog and bear described by Wineza may be similarly explained.

The conclusions reached therefore are: 1) the reduction or the absence of a medial part of the alar element of the cat, comparable with the origin of the anterior process from the sella turcica of mole; 2) the presence of a simple processus alaris in the cat, of a compound one in the mole; 3) the persistence of the original boundary between the lateral and commissural elements in the cat and the obliteration of the limits between the comparable anterior and middle processes in the mole. It follows that the ala temporalis of mole and cat are in the main comparable. It is probable that other examples of the types of continuous and independent temporal wings may be similarly explained.

Whether the lamina ascendens of the cat should be regarded as an independent element in origin, or continuous with the basis cranii, is a question which could be answered either way from the evidence here presented, and would be purely a choice of interpretations. The whole ala temporalis is shadowed in mesenchyma, continuous with the basis cranii; in this adumbration condensations appear which are distinct from one another or confluent at the edges, as you choose to describe the conditions. Comparison of the lateral element of the cat with the anterior process of the mole inclines me to regard this element and its derivative, the ala temporalis, as not an independent element genetically. There appear to be two parts under consideration in comparisons of the ala temporalis of different mammals, (1) a part which is related chiefly to the carotid artery, represented by the middle process of the mole and the commissural process of the cat; (2) a part which is related to the semilunar ganglion and the three divisions of the fifth nerve. These two parts are typically both connected with the cranial base, the one arch-like, the other process-like. Both are typically originally separate from one another, the process standing in front and at the side of the arch. In some forms the process unites with the arch completely (mole), in others partially (hedge-hog), in still others not at all (polar-bear, cat). Finally, this conception of the comparison of the anterior process of mole and the ala temporalis of cat, supports the homology of the ala temporalis of placental mammals with the ala temporalis of *Echidna*.

*Pterygoid.* The pterygoid cartilage is developed relatively late, not until after bone has begun to be formed in its dorsal part. It is represented by a rather ill-defined mesenchymal condensation, even at the time when the ala temporalis is well chondrified. Only its caudal part is cylindrical and thus bears some resemblance to the cartilage of the medial lamella of the pterygoid process in *Talpa*. It is unlike this process in not reaching forward to the ethmoidal region. However, as pointed out, it develops in the same morphological plane as the palate bone, lies next the ductus nasopharyngeus, in front of the first pharyngeal pouch and is crossed dorsad by the Vidian nerve.

Jayne ('98) has described, in the skull of the adult cat, a pterygoid process presenting a well defined internal pterygoid plate or process and a variable external plate or process. Union of the originally separate pterygoid bones with the alisphenoid takes place very early, and sutures or lines indicating their boundaries cannot be found in the adult. The pterygoid elements are represented therefore, as processes of the alisphenoids. Reighard ('02) describes an external pterygoid muscle, taking origin from the external pterygoid fossa whose surface includes the lateral aspect of the external pterygoid plate of Jayne, and an internal pterygoid muscle springing from the internal pterygoid fossa; the latter is bounded in part by the medial surface of the external plate. The internal pterygoid plate terminates in a hamular process, related, in the usual manner, to the tendon of the tensor palati. The origin of these two bony processes was found in the present study in cat embryos of 7 cm., and 23.1 mm., the external process being an extension of the endochondral ossification of the pterygoid process of the ala temporalis, the internal process consisting of an ossification, at first in membrane and subsequently in cartilage, in connection with the separate pterygoid cartilage. The latter accords with an early stage of the human internal pterygoid plate as described by Fawcett ('10), both in its early ossification (it is the first part of the sphenoid to ossify in the cat) and in the ossific process, proceeding primarily in membrane and later in cartilage. The external plate is feebly developed in the cat, but its ossification in relation to that of the ala temporalis is nevertheless, in principle, the same as in man. It will be remembered that the Vidian nerve runs along the mesenchymal junction of the pterygoid cartilage and pterygoid process of the ala temporalis; now, although no suture or line can be found in the adult skull indicative of the original limit of the pterygoid bone toward the alisphenoid, as Jayne has stated, yet the course of the bony walled Vidian canal of the adult can be taken as marking this boundary.

*Carotid foramen.* The epipteric cavity in the cat embryos is limited, toward the primary cranial cavity, by a membrane whose relations to the base of the skull are of considerable interest as



affecting the question of the nature of the region about the carotid artery. In *Echidna* the carotid enters directly the primary cranial cavity, but the position of the foramen through which it passes lies within the lateral confines (*trabecula basis cranii*) of the hypophyseal fossa and therefore may be compared with the entrance into the cranium of this vessel in *Lacerta*. In placental mammals the carotid foramen lies laterad of the sella turcica, a position which has been explained by two assumptions: (a) lateral migration of the vessel, (b) non-equivalency of the mammalian and reptilian carotid. In accordance with the theory of lateral migration, the carotid foramen is supposed to have moved outward across that part of the cranial floor equivalent to the trabecula; or both the trabecula and artery have moved lateral while still retaining their primitive relations to each other. The trabecula cranii of *Lacerta* is represented in mammals, according to Gaupp ('02), by the aliochlear commissure. The unity of this commissure, including both the processus alaris and commissura aliochlearis, its distinction from the ala temporalis, and its relation to the base of the cranium were recognized by Gaupp. In one place ('06), he says: "The processus alaris of man *appears* as a process only through the decadence of the cartilaginous bridge which closes laterally the carotid foramen. It belongs to the median cartilaginous mass in the base of the orbito-temporal region." Voit, who has advocated the view of non-equivalency of the internal carotid in mammal and reptile, locates the carotid foramen in *Lepus* laterad of the trabecular region. He concludes that the commissura aliochlearis plus the processus alaris alae temporalis should not be compared with some part of the trabecula, but rather the processus alaris should be referred to the processus basiapterigoideus of *Lacerta* and ala temporalis of *Echidna*. The commissura aliochlearis is regarded by Voit as a new structure, in mammals, in the floor of the epipteric cavity, a continuation of the floor of the cavum supracochleare. Fuchs ('10) also, compares the medial part (root) of the ala temporalis with the processus basiapterigoideus of reptiles. In accordance with Voit's interpretation, the carotid artery first enters the epipteric cave, then, after traversing the medial limiting membrane



of the cave, comes into the primary cranial cavity. Recently, De Burlet ('13) has brought forward evidence of the carotid in *Phocaena* traversing the trabecula or its equivalent. In the porpoise the carotid passes directly into the primary cranial cavity. As we have seen, the limiting membrane in the cat is fixed to the basis cranii, neither to one or the other side of the carotid foramen, but broadly over the region where the vessel enters. It is attached both to the aliochlear commissure and to the lateral margin of the sella turcica. The former does not, in the cat, enter into the floor of the *cavum epiptericum*, nor does the carotid artery pass directly into the primary cranial cavity. The vessel first traverses that part of the *membrana limitans* which is fixed to the cranial floor, before passing into the original cavity of the skull. As to the homology of the *commissura aliochlearis*, it is evident that if the *processus alaris* is not included as a part of it, the difficulty of comparing the commissure with the *trabecula cranii* is much increased; if the alar process be recognized as the continuation of the aliochlear commissure forward, in continuity with the median basal cartilage of the orbito-temporal region, the comparison is far less difficult. Reviewing the conclusions stated above, we may note, first, that the name *commissura aliochlearis* is a misnomer, since it does not express the true relation of the commissure, but implies a connection with the *ala temporalis* which does not exist in the mammals so far studied. Secondly the studies of Noordenbos on the mole and my own on the cat show that the so-called *processus alaris* may be simple or compound in different animals, but that it is primarily a part of the *commissura aliochlearis* (its anterior end in fact), continuing into the median basal cartilage; relation of the *processus alaris* to the *ala temporalis* is secondary and obtains in those types (mole) where the *ala* springs from the basal cartilage; its root, at first separate from, later becomes fused with the *processus alaris* to produce the compound form of alar process. When the *ala temporalis* is independent of the basal cartilage (cat) the former has nothing to do with the *processus alaris*, which then is the simple extension forward of the *commissura aliochlearis* into the *sella turcica*.

A word may be said regarding the membranous structure here called septum transversum. The development, in its basal part, of a marked thickening which extends from the crista transversa to the parietal plate, together with the presence in it of a cartilaginous bar over the semilunar ganglion are suggestive of structures in the reptilia, such as Shino ('14) has described in the crocodile and compared with the pila prootica of *Lacerta*. A cartilage over the semilunar ganglion in *Lepus* recorded by Voit was interpreted as a vestige of the primary cranial wall; in the cat, a cartilaginous mass, having the same position and relations, was found within the septum transversum.

*Ala orbitalis*. The occurrence generally, in a wide range of types, of the independent origin of the ala orbitalis has been emphasized by Noordenbos. This phenomenon, as shown above, is also characteristic of the cranial development of the cat. The idea of the association of the ala orbitalis, primarily with the optic nerve and eye-ball, seems to be well founded and receives further support from the early form and relations of this cartilage in the cat. The relations of the origins of the ocular muscles may be interpreted as further indicating a close connection between the orbital wing and the organ of sight. The definitive origins of these muscles are in the main related to the optic foramen, about as they are in the chondrocranium, but some shifting has evidently taken place as comparison of the two states shows (Wilder and Gage '86; Corning '02). Four definite separate spots in the chondrocranium are occupied by, and one of them apparently specially adapted to, the attachment of the muscles of the orbit. How these attachments compare with those in the mammalian types represented in the literature of the chondrocranium I cannot say, since this question does not seem to have received attention. Does the superior oblique constantly spring from the preoptic root; does the orbital process of the metoptic root function generally as a point of attachment of a definite group of eye-muscles; is the side of the basis cranii in the orbital fissure a special locus of origin of another group, and is the origin of the inferior oblique constantly the planum antorbitale? Regarding the metoptic root, it has been noted that, in contrast to the

straight direction of the preoptic, it presents a marked bend, convex caudally. This curve is apparent to a slight extent in the bony cranium. Judging from figures of the chondrocranium of several mammals, the bend in question seems to be a characteristic of this cartilaginous rod. In cat the knuckle of the curve apparently mark the place of union of the metoptic process of the trabecular plate and the corresponding process of the ala orbitalis. Opposite this spot the oculo-motor nerve leaves the cranium by the orbital fissure. What significance there is in the form and relations of the metoptic root, which are early established and permanently retained, must await future inquiry.

### *Ethmoidal region*

*Region of the olfactory fenestra.* The floor of the chondrocranium of mammals, between the levels of the preoptic root of the ala orbitalis and the fenestra olfactoria, is made by the lamina infracribosa, which is the roof of the posterior cupola of the nasal capsule and an extension of the planum antorbitale. The lateral branch of the naso-ciliary nerve runs upon this lamina on its way toward the interior of the nasal capsule, having come through the sphenothmoidal fenestra from the orbit. The lamina cribrosa, a structure peculiar to mammals, develops over the fenestra olfactoria. Now, in reptiles the ethmoidal nerve and the whole of the posterior cupola, with the olfactory fenestra, are extracranial. A study of these contrasting conditions of the mammalian and reptilian ethmoidal skeletons has led Gaupp to the conclusion that a new region, named by him recessus supracribrosus, has been added to the mammalian brain case, and that this region is comparable, in reptiles, with the extracranial parts about the olfactory fenestra. In the cat, the form and relations of the posterior cupola and olfactory fenestra are essentially as in other mammals. The fenestra olfactoria opens directly into the chondrocranial cavity. As already stated, the lamina cribrosa was not present in the stages of the cat studied, consequently no data have been presented bearing on the question of its position relative to the walls of the chondrocranium. The

lamina infracribrosa is a part of the antorbital division of the nasal capsule, entering into the floor of the cranium. The course of the ethmoidal nerve is, strictly speaking, outside the chondrocranial cavity, since the layer of mesenchyma (probably in part giving rise to dura), which covers the lamina infracribrosa, stands between it and the cerebral cavity. In the cat, in the stages studied, there is no overhanging anterior wall of the chondrocranium such as is shown in the model of *Echidna*; a flat anterior margin of the fenestra olfactoria marks the anterior limit of the cranial cavity. Consequently, the term 'recessus' supracribrosus is hardly appropriate for the cat; the term 'supracribrous region' is preferable. From these data, it seems that the ethmoidal region of the cat, like the orbito-temporal and otic regions, gives evidence in support of Gaupp's assertion of the non-equivalency of reptilian and mammalian crania, and also, by its relations to the chondrocranial cavity, indicates that the latter is enlarged over that of reptiles by the acquisition of a region wholly extracranial in that class. A factor having a large share in bringing about this mammalian characteristic is the backward growth of the nasal cavity (Weber, '04; Gaupp, '08) whereby the interorbital septum is encroached upon by the posterior cupola of the ethmoidal skeleton. That this occurs to a slight extent in the ontogeny of the cat was indicated by the difference in position of the posterior cupola relative to the preoptic process in earlier and later embryonic stages.

In *Lepus*, according to Voit, the foramina of the lamina cribrosa are separated into two groups by a crista intercribrosa, olfactory filaments from the recessus lateralis traversing the foramina of the antero-lateral group, those from the recessus posterior (ethmoturbinal region) passing through the holes of the postero-medial group. Voit also found the dorsal end of ethmoturbinal I continuous with the crista intercribrosa. Continuity between these two parts is present in *Caluromys philander*, as observed by Dr. Denison in this laboratory. In the cat, at the stage modeled, the dorsal end of ethmoturbinal I is very prominent and stands between the group of olfactory filaments coming from the epithelium of the recessus lateralis and those from the ethmo-



turbinal region. The latter group is further subdivided by the dorsal end of ethmoturbinal II into two bundles coming, one from the area in front of, the other from the area behind this turbinal process.

*The floor of the nose and Jacobson's cartilage.* In cat embryos of the stage modeled, the secondary palate is nearly complete, forming a floor for the nasal cavities. The latter are separated from each other by the septum nasi, excepting for a stretch posteriorly, where the ventral margin of the nasal partition is free from and above the level of the palate. Between the maxilloturbinal dorsally and the nasal floor is a broad groove of the lateral nasal wall, which extends from before backward to the choanae. This groove, the inferior meatus of adult anatomy, the lower furrow of Legal ('83) or the choanal passage of Fleischmann and Beeker ('03), besides differing from the other nasal passages by its origin from the primitive mouth, is specialized through its relation to the naso-lacrimal duct and Jacobson's organ. A striking feature of this ventral part of the *cavum nasi* is the variability among mammals of its cartilaginous walls and floor. This characteristic stands in marked contrast to the usual condition of stability of the dorsal part of the ethmoidal skeleton (Spurgat, '96).

The peculiar characteristics of the nasal floor cartilages in the cat have been described by several investigators. Our observations must be regarded as confirmatory of Broom's ('96) description and of Zuckerkandl's ('08) more recent representation of the paraseptal and related cartilages. Both of these authors found an inner and outer division or process of the nasal floor cartilage (*lamina transversalis anterior*) and a cartilage related to the nasopalatine duct.

The significance and homology of these parts of the ethmoidal skeleton have been much discussed. Division of the *lamina transversalis anterior*, into medial and lateral cartilages (inner and outer parts of the nasal floor cartilages) embracing the nasopalatine duct, has been observed by Broom ('96) to occur with considerable constancy in the mammalian series. The outer portion of the nasal floor cartilage stands lateral to the nasopalatine



canal. In *Ornithorhynchus* and *Echidna* this cartilage expands at its caudal end and unites with its fellow across the midline to form a broad plate beneath the organ of Jacobson and its cartilaginous capsule. The conditions in the Marsupialia were traceable to those of the monotreme type. In *Lepus*, as representing the rodents, Broom found an independent cartilage supporting the outer side of the nasopalatine canal, which was homologized with the outer nasal floor cartilage of the Monotremes, and was regarded as a much modified form, compared with the simple higher eutherian type. Voit has also described, under the name *cartilago nasopalatina*, the supporting cartilage for the nasopalatine duct in the rabbit, and, with Gaupp, compares it with the *processus palatinus* (outer nasal floor cartilage of Broom) of *Echidna*.

This comparison is founded by Voit mainly on the fact that in certain mammals (horse, pig and sheep) the *processus palatinus* (*cartilago basalis lateralis* as described by Spurgat, '96) is united with the *lamina transversalis anterior* (*processus septi cartilaginei lateralis ventralis*) in the same relationship as is the case with the *processus palatinus* of *Echidna*; and further, that in at least one form, *Vesperugo noctula*, described by Grosser ('02) the '*cartilaginee posteriores laterales*' (*cartilaginee nasopalatinae* of Voit) are considerably broadened posteriorly and fused across the median line, as is true for the palatal processes of *Echidna*.

Two conditions, presented by the cat, seem to indicate that the significance of the nasopalatine cartilage and the *processus posterior lateralis* of the *lamina transversalis*, is not fully brought out by the above comparisons. These conditions are (a) the presence, at the same time, of two separate cartilages, one in relation to the nasopalatine duct (nasopalatine cartilage) the other in the floor of the nose next to the entrance of the nasopalatine duct (posterior lateral process of the transverse lamina); and (b) the independent origin of the former. Furthermore, in regard to the first condition, it seems evident, from Grosser's description of *Vesperugo*, that both paired nasopalatine cartilages (*cartilago ductus incisivi*) and posterior lateral cartilages are present and separate, the one pair from the other.

A cartilage, having the position and relations to the nasopalatine duct and to the anterior transverse lamina, such as presented by the posterior lateral cartilage of *Vesperugo* and the lateral process of the lamina transversalis anterior of *Felis*, is of frequent occurrence in the mammalian series. It stands as a support to the floor of the nose at the outer side of the entrance to the nasopalatine duct and probably possesses, as Grosser says, a great significance as a supplementary structure to the palate. It is found in forms in which a nasopalatine cartilage (as here defined) does not exist, notably in mammals having a very short nasopalatine duct (*Echidna*) and also in forms where the nasopalatine cartilage is present, in which cases it may be separate from or united with the latter.

In accordance with this view, the processus palatinus of *Echidna* would be comparable with the posterior lateral cartilage of *Vesperugo*, the lateral process of the lamina transversalis anterior of *Felis*, and not with the cartilago ductus incisivi of these forms. The primary relation of the nasopalatine cartilage (cartilago ductus incisivi), on the other hand, appears to be directly with the nasopalatine duct, as in the cat. There is some evidence in the series of mammals of its correlation with the presence of a long nasopalatine canal. Absence of a nasopalatine cartilage in the lower mammals and its independent origin and late appearance in development in the cat may be interpreted as indicating that it is phylogenetically a recent acquisition to the palatal skeleton, and also that its connection with the lateral posterior cartilage may be regarded as secondary.

Jacobson's cartilage, relatively short, arises independently, as recorded by Schwink ('88) and as observed in the present study, and remains ununited with other parts of the chondrocranium in embryos of the cat up to 23.1 mm. In later stages, as Zuckerkandl has observed, this cartilage has elongated and taken on connections with the lamina transversalis posterior, through the development of a slender paraseptal rod. Zuckerkandl believed that Jacobson's cartilage represents the anterior half of a cartilaginous ridge which is derived from the primitive floor of the nose. This is in accord with Seydel's ('99) conception of the

paranasal cartilage forming a part of the primitive nasal floor which, by reduction, came eventually to be represented by more or less separate parts (Jacobson's cartilage) in the mammals. In the light of this interpretation, the slender cartilaginous parasseptal strip which appears late in the development of the cat, and unites Jacobson's cartilage with the posterior transverse lamina, may be regarded as evidence of a partial restoration of the primitive floor.

*Tectum nasi.* Noordenbos has observed the beginning of the tectum nasi in mole embryos of 9 mm. as bilateral divisions of the anterior margin of the trabecular plate, forming curved lamellae. In the dorsal middle line a shallow groove stands between the two halves of the tectum. In the cat the nasal tectum arises apparently similarly in connection with the nasal septum, as the parieto-ectal cartilages, although, as already mentioned, some evidence of the independent origin of these cartilages was presented. The groove between them, dorsal of the septum, is the beginning of the sulcus suprasedalis. In the cat, the parieto-ectal cartilages grow back, along the dorsal margin of the septal cartilage, as far as the fenestra olfactoria, becoming gradually narrower from side to side and assuming the form of triangular plates. These cartilages contribute the entire roof of the precerebral division of the nose and the side wall anterior to the recessus lateralis. What the relations of the parieto-ectal are to the development of the lamina transversalis anterior were not determined. Eventually a complete zona annularis is established behind the level of the fenestra narina, the roof being derived from the parieto-ectal cartilage, the floor from the lamina transversalis anterior, the lateral wall from the union of these two parts. The relations of the lateral margin of the parieto-ectal with the anterior free margin of the paranasal cartilage are at first similar to those in *Talpa*, as observed by Noordenbos. This cartilage does not seem to be concerned in the formation of the maxilloturbinal, although in the stage of 23.1 mm. this process is connected with the anterior part of the lateral nasal wall through the lamina supraconchalis. Within the nasal cavity the atrioturbinal appears as the inrolled ventral margin of the parietotectal cartilage.

*Paranasal cartilage.* Independent origin of a plate of cartilage lying in the anterior part of the lateral wall of the nose has been observed by Noordenbos in embryos of *Talpa* of 9 mm. length; also in the embryos of calf and pig. Following Mihalkovics, the cartilage was called cartilago paranasalis. In *Talpa*, the paranasal cartilage and the tectum nasi are separated by a fissure, bounded by the anterior border of the former and the free margin of the latter. In the cat, also, an independent paranasal cartilage occurs which bears similar relations to the parieto-tectal cartilage. It is directly over that diverticulum of the cavum nasi which later is included in the recessus lateralis of the ethmoidal skeleton. The little cartilaginous plate, bent upon the convex bulging of the nasal sac, presents free margins: one toward the side of the olfactory lobe; one backward toward the eye; a ventral margin, and a long anterior side, opposite the free, oblique edge of the parieto-tectal cartilage; relations comparable with those observed in the mole.

Great interest attaches to the ventral and anterior margins of the paranasal cartilage. The latter overlaps the parieto-tectal, and, whereas in younger embryos it is separated from that cartilage by a fissure, it is later fused with it. The result of this fusion is a curved, intranasal ridge, the crista semicircularis, perforated by the foramen epiphaniale, which is the remains of the original fissure. Thus is explained the position and extent, quite to the olfactory fenestra, of the semicircular crest. It follows from its mode of formation that, in later stages, the crista semicircularis may be taken as the boundary between two originally distinct territories of the nasal wall. During the period occupied by the process of fusion of the incurved anterior margin of the paranasal and the hinder margin of the parieto-tectal cartilage, and for some time thereafter, an interval of variable extent (derived from the space of the original separating fissure) is enclosed within the encompassing cartilaginous margins; that is, for a time the crista semicircularis presents double walls with an intervening space; this space is crossed by the lateral nasal nerve at the level of the future epiphanyal foramen.



Now in the nasal capsule of *Lacerta*, at the place where the *zona annularis* joins posteriorly the broader, bulging portion of the side wall, a conchal fold is present, projecting into the nasal cavity; within the *aditus conchae*, or entrance to the conchal fold is the foramen for the lateral nasal nerve. The fissure-like *aditus* extends dorso-ventrad, its convex lateral margin overhanging the medial margin made by the *zona annularis*. Gaupp says of it ('00, p. 484): An der Muscheleinstülpung sind zwei Lamellen, eine mediale und eine laterale, zu unterscheiden. Die mediale Lamelle der Concha liegt in der direkten Fortsetzung der Seitenwand der vorderen Nasenkapselhälfte, sie endet ventral mit freiem Rande. . . . Die laterale Lamelle der Concha geht ventralwärts in den Boden des Recessus extraconchalis über.

The *aditus*, in *Lacerta*, lodges the external nasal gland; its duct passes forward across the outer side of the *zona annularis* to enter the nasal mucosa through a gap in the side wall of the nasal capsule, behind the *processus alaris superior*. Finally, the concha itself forms a ventral and anterior partial partition between the general space of the nasal capsule and a lateral *diverticulum* which Gaupp has named the *recessus extraconchalis*. The incomplete floor of the recessus is made by the ventral prolongation of the lateral lamella of the concha.

It seems evident that in the stage of development of the ethmoidal skeleton in cat when fusion is occurring between the parieto-tectal and paranasal cartilages, certain conditions are presented which reflect the structure of the reptilian nasal capsule, as shown by the embryo of *Lacerta*. The general direction of the space between the parieto-tectal and paranasal cartilages on the one hand, and the *aditus conchae* on the other, is the same, and its boundaries are made by lamellae similarly placed in respect to each other; it is traversed by the lateral nasal nerve in both forms. The position of the external nasal gland in the *aditus conchae* of *Lacerta* and of the externally coursing duct is, apparently, impossible to reconcile, on our present knowledge, with the lateral nasal gland of *Felis* and the intranasal course of its duct, if these glands be comparable at



all. Peter ('06) seems to regard the mammalian gland as a new acquisition. It will be remembered, however, that the lateral nasal gland in the cat embryo of 23.1 mm. is located just in front of the base of the crista semicircularis.

Next, we may attempt to compare that part of the nasal wall (paranasal cartilage), which stands just behind the fissure in the cat, and the aditus conchae in lizard. We have seen that the commissura spheno-ethmoidalis unites, in cat embryos of 17 mm., with the dorsal margin of the paranasal cartilage, where the latter bounds the olfactory fenestra, thus presenting relations which recall those between the posterior half of the side wall of the nasal capsule and the cartilago spheno-ethmoidalis of *Lacerta*. For, at the junction of these parts, in the cranium of the lizard, the lateral wall limits by its upper margin the olfactory fenestra while, medially, it passes into the posterior wall, the planum antorbitale, as is also true for the cat. It has been noticed that the ventral and anterior margins of the paranasal, are continuous inrolled edges, which form the cartilaginous maxillo-turbinal and, with the free posterior margin of the parieto-tectal cartilage, form the beginning of the crista semicircularis. In *Lacerta*, the part of the side wall in question goes over anteriorly into the lateral overhanging plate of the bilaminar concha, the medial plate of which is the direct continuation of the side wall of the anterior half of the nasal capsule. Furthermore, the lateral plate passes ventrally into the floor of the recessus extraconchalis. Of great interest is the resemblance between the concha and floor of the recessus extraconchalis of *Lacerta* on the one hand and the crista semicircularis and maxillo-turbinal of the cat on the other.

This comparison brings out a certain degree of similarity of form and relation between the skeletal parts of the region of the recessus extraconchalis of *Lacerta* (embryo of 31 mm. total length) and of the recessus lateralis of the cat (embryos of 17-20 mm.). An opinion as to a possible homology between these parts should not be ventured on a comparison limited to the data here presented. Greater knowledge of the development of the ethmoidal skeleton in the lizard is required, and additional

data on the history of the paranasal cartilage in mammals is essential.

As to the origin of the lamina supraconchalis and the broad base of the naso-turbinal, unfortunately little evidence was found. The region of the side wall occupied by these parts corresponds to that between the antero-inferior quarter of the paranasal and the adjacent region of the parieto-tectal cartilage. I cannot say to what extent, if at all, these two cartilages enter into the lamina supraconchalis and naso-turbinal.

One other matter should be mentioned with which, possibly, the paranasal cartilage may be related—the origin of the inferior oblique muscle. In the adult cat, this muscle arises from the orbital surface of the maxilla behind the lacrimal bone (Reighard, Jayne, Corning). In the embryo of 23.1 mm. its origin is from a prominent angle of the lateral nasal wall, at the junction of the paries nasi and planum antorbitale. In the 17 mm. embryo this angle corresponds with the ventral inferior corner of the paranasal plate, overlapping the anterior margin of the antorbital plate. Now, although no posterior maxillary process is developed in cat in the stages studied, yet the angle here referred to possibly represents this process. I have not noted in the literature the origin of the inferior oblique from the chondrocranium in mammals. Corning ('02), quoting Weber on its origin in *Lacerta viridis*, says (p. 122): "Der M. obliquus inferior 'entspringt von der Cartilago ethmoidalis, wo diese sich an den Knochen der vorderen Orbitalwand ansetzt.'"

*Lamina antorbitalis.* Noordenbos is apparently the only one who has observed in mammals the isolated origin of the cartilage forming the posterior part of the nasal capsule. This element of the ethmoidal skeleton, found in mole embryos of 10.5 mm. and 11 mm., finds its parallel in the cat, where, as stated, a separate plate of cartilage forms the planum antorbitale and cupola posterior. Union between the antorbital plate and the paranasal in the cat must take place rapidly, as no trace of an interval between the two parts is to be seen in embryos of 20 mm. Complete union, that is, fusion, with the septal cartilage is not present in the embryo of the stage modeled. Of much in-

terest is the relation of the antorbital plate to the ethmoturbinal bodies. A well developed, epithelial ethmoturbinal fold can be seen behind the recessus lateralis in embryos of 15 to 17 mm. We have seen that the posterior margin of the paranasal cartilage overlaps the anterior edge of the antorbital plate, and that the latter projects forward and medialward into the base of the epithelial fold of ethmoturbinal I. Sections show that the anterior, free cartilaginous extremity of this ethmoturbinal, in embryos of 23.1 mm., is formed, in connection with mesenchyma, within the fold of epithelium and independently of the wall of the nasal capsule. The mesenchyma can be followed, however, backward to the base of the fold and to the ridge of cartilage derived from the antorbital plate of earlier stages, which bounds posteriorly the opening into the recessus lateralis.

The development of the ethmoidal skeleton in the cat indicates a certain degree of correlation between its cartilaginous components and regions of the nose more or less sharply defined by particular functions. The dorsal, larger, and more completely walled division includes the olfactory and paranasal territories and the atrium opening toward them; whereas the ventral, smaller and imperfectly formed division stands in relation to the respiratory air passage, nasopalatine and lacrimal ducts. Even the original components of the dorsal division present evidence of specific adaptation to functional territories. Thus, the antorbital plate forms the wall of the ethmoturbinal territory of distribution of the olfactory nerve, the paranasal cartilage incloses the diverticulum of the lateral recess, later the frontal sinus; and the parieto-tectal develops into the wall of the atrium. I do not wish to imply that the evidence at hand justifies the conclusion of rigorous specificity of these components, it certainly does not; but facts are sufficient to indicate that each of these original cartilages is, in the main, developed in relation to recognized functional territories of the nose.

## SUMMARY

1. The basal plate of the occipital region in the cat is derived from a pair of parachordal cartilages and from two, probably three, hypochondral commissures or arches. The parachordals are continuous with the lateral occipital arches and, topographically, are to be compared with the lateral masses of the atlas. Chondrification of the perinotochordal sheath in the occipital region forms the apex of the dens epistrophei, which, in certain respects, is comparable with a vertebral centrum.

2. The observations on the developmental processes of the occipital region in the cat indicate that, in principle, they are comparable with those of an atypical vertebra of the atlas kind. The basal plate of the occipital region falls into the category of arch structures, not centra.

3. In the atlas, the beginning of an atlantal foramen is very early indicated by a notch, traversed by the first spinal nerve, the sides of which are formed by the neural arch and a process of the lateral mass. The possible significance of this atlantal process was discussed.

4. The occipital condyles are located primarily on that part of the basal plate derived from the parachordal cartilages and, in accordance with the interpretation of the form of the occipital element, belong to arch structures and not to centra.

5. The position of the lateral occipital arches and the plane of the foramen magnum, parallel with the floor of the nose in embryos of 23.1 mm., is correlated with flexures, in a sagittal plane, of the cranium as a whole.

6. The fissures around the otic capsule, between it and the basal plate and walls of the chondrocranium in the 23.1 mm. embryo, are vestiges of the spaces primarily existing between these independently arising cranial elements.

7. The incisura occipitalis posterior of the cat embryo is intermediate in relative size between that of the reptile (*Lacerta*) and that of man. There is apparently a tendency for this space to increase in area from lower to higher forms.

8. The upright position of the axis of the otic capsules in cat embryos of the stage modeled is exceptional for mammals. There is evidence in the literature that the obliquity of the otic axis characteristic of the mammalian chondrocranium is attained at somewhat different periods of development in different species.

9. The origin of the cartilaginous pars canicularis of the otic capsule is independent of other parts of the cranium. The pars cochlearis arises in connection with the pars canicularis and the suprafacial commissure. The latter is continuous with the orbito-parietal commissure, and is therefore, in part at least, to be regarded as a parietal structure. The cochlear capsule forms independently of the basal plate, which in the cat is reduced to a narrow bar in the otic region. The observations on the relationship of the cochlear capsule to the basal plate and suprafacial commissure are interpreted to support Gaupp's theory on the development of the cochlear capsule and reformation of the cranial base.

10. A single acoustic fissure, transmitting the two divisions of the acoustic nerve, is situated at the bottom of a shallow internal acoustic meatus. The latter results, principally, from the elevations of the suprafacial commissure and commissura basi-vestibularis, these forming its anterior and posterior walls.

11. The fenestra cochleae and aquaeductus cochleae result from the division of the foramen perilymphaticum by a process extending from the cochlear capsule.

12. The cavum vestibulare of the cartilaginous otic capsule of the cat is comparable with that of the reptile (*Lacerta*) in possessing two subdivisions, a separating partition and similar relations to parts of the membranous labyrinth.

13. The lamina parietalis seems to be derived from two independently arising centers of chondrification, an anterior one, which also constitutes the orbito-parietal commissure and unites with the otic capsule in the parietocapsular commissure; and a posterior center which unites, first, with the summit of the lateral occipital arch and, next, with the pars canicularis. From the posterior center (called parietal plate in the descrip-



tion) the tectum posterius apparently arises as a process. The definitive parietal plate seems to be the result of the fusion of the anterior and posterior elements.

14. The facial nerve makes its exit from the chondrocranium by the primary facial foramen, at which spot is located the geniculate ganglion and the origin of the great superficial petrosal nerve. The primary facial foramen is to be regarded as comparable with the foramen faciale of *Lacerta*.

15. The floor of the hypophyseal fossa is formed from a single crescentric cartilage arising independently.

16. In the cat a small space, filled with mesenchyma, existing for a brief period, between the anterior end of the basal plate and the hypophyseal cartilage, is identified as a fenestra basicranialis posterior.

17. The dorsum sellae in cat is formed from the crista transversa and, in addition, by chondrification of the mesenchyma dorsad of it, probably by a separate center. There is evidence in the literature that the dorsum sellae of the lower mammals is a derivative of the crista transversa alone, while that of the higher mammals includes an additional element.

18. The development of the ala temporalis of the cat, representing the type of discontinuous ala, is comparable with that of mole, representing the type of ala temporalis continuous with the sella turcica. The difference between these forms of ala is an apparent one, resulting from failure of the root of the temporal wing of the cat to form in cartilage. The synchondrosis between the ala temporalis and aliochlear commissure and the incisura lacera of cat, the foramen or fissure between ala and commissure in other forms, indicate the plane of junction between these originally independent and different parts. The ala temporalis of cat is comparable with the ala temporalis of *Echidna*.

19. A pterygoid element appears in the cat as a condensation of mesenchyma, in which ossification is occurring at the stage of the 23.1 mm. embryo. Cartilage is subsequently developed, in which ossification proceeds. There is evidence of two ossific centers, and from these the medial pterygoid lamella and hamular process are formed.

20. A *cavum epiptericum* and *cavum supracochleare* are recognized in the chondrocranium of cat. The names '*membrana limitans*' and '*septum transversum*' are proposed for the mesenchymal sheets connected with the walls of the orbito-temporal region. In the cat the internal carotid artery enters the *membrana limitans*, by the carotid foramen, and secondarily into the primitive cranial cavity. A bar of cartilage in the *septum transversum*, lying upon the semilunar ganglion, is probably a vestige of the primitive chondrocranial wall.

21. The *ala orbitalis* arises independently in the cat and unites secondarily with the paranasal cartilage, orbitoparietal commissure, and trabecular plate. It is apparently primarily adapted to the eyeball and its adnexa.

22. A region, extracranial in reptiles, is represented within the chondrocranium of cat as the *supracribrous* region of the ethmoidal skeleton.

23. The structure of the anterior part of the floor of the nose is in agreement with the descriptions of Broom and Zuckerkandl. The nasopalatine cartilage of mammals is interpreted as a structure originally distinct from the lateral posterior cartilage of the nasal floor, and its connection with the latter secondary.

24. Jacobson's cartilage in the cat is the anterior, well developed, portion of a paraseptal cartilage, connected posteriorly with the *lamina transversalis posterior*.

25. The cartilaginous ethmoidal skeleton of the cat is separated on developmental grounds and physiological relations, into a small ventral part and a large dorsal part. The former presents incomplete walls about the inferior meatus; the latter rather continuous walls about the paranasal sinuses and olfactory region of the nose.

26. The dorsal part of the ethmoidal skeleton in embryos to the stage of 30 mm. is formed from the septal cartilage, parieto-tectal cartilages, paranasal cartilages and the *laminae antorbitales*.

27. The parieto-tectal cartilages, paired, spring from the septal cartilage. They contribute the roof of the precerebral division of the ethmoidal skeleton, the side wall of the atrial region,

participate in forming the crista semicircularis and probably, to some extent, give origin to the alar cartilages.

28. The paranasal cartilages, paired, arise independently and are primarily adapted to the diverticula of the recessus laterales. They participate with the parieto-tectal cartilages in the formation of the semicircular crests and in the origin of the maxillo-turbinal processes. From this cartilage the inferior oblique muscle of the eye arises.

29. The foramen epiphaniale is the vestige of the original space between the parieto-tectal and paranasal cartilages.

30. The lamina antorbitalis, paired, arises independently, and unites secondarily with the paranasal and septal cartilages. From it are formed the posterior cupola and the bases of the ethmo-turbinals I and II.

31. The ethmoidal skeleton of cat may be compared with that of *Lacerta* as follows: the region of alar cartilages in the cat and the anterior zone of the lizard; tectum nasi and lamina transversalis anterior of the cat with the zona annularis of the lizard; the combined paranasal and antorbital parts of the cat with the posterior zone of the saurian.

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## EXPLANATION OF FIGURES

All figures are from preparations of embryos of the cat, the lengths of which are indicated. With the exception of figures 17 and 18, the drawings are the work of Mr. C. D. Jarrett.

In the model from which figures 1 to 5, 12, 13 have been made, cartilaginous parts only are represented on the left side; on the right side, in addition, are shown vessels, nerves and bones. In figures 1 and 2, the right otic capsule is represented as partly opened. In figure 3, the incudal cartilage and part of Meckel's cartilage have been omitted. Figures 15 to 30 are camera lucida drawings of the sections represented. Figures 19 to 30 are from sections of the 23.1 mm. embryo (H. E. C. Series 466); the number of the section is indicated in each figure.

Fig. 1. From a model of the cranium of an embryo 23.1 mm. long (H. E. C. Series 466) Dorsal aspect,  $\times \frac{1}{1.5}$ .

Fig. 2 The same; ventral aspect.

Fig. 3 The same; left lateral aspect.

Fig. 4 The same; right lateral aspect.

Fig. 5 From a van Wijhe preparation of the head of an embryo 10.5 mm. long (W. A. C. No. 310). Dorsal aspect,  $\times \frac{2}{3}$ .

Fig. 6 From a van Wijhe preparation of the head of an embryo 12 mm. long (W. A. C. No. 311). Left side and base seen from the right,  $\times \frac{1}{1.3}$ .

Fig. 7 From a van Wijhe preparation of the head of an embryo 15 mm. long (W. A. C. No. 312). Dorsal aspect,  $\times \frac{1}{1.3}$ . The breadth of this is somewhat exaggerated, the ala temporalis being too far toward the side.

Fig. 8 From van Wijhe preparations of the heads of embryos 18-20 mm. long (W. A. C. Nos. 313, 314). Dorsal aspect,  $\times \frac{1}{1.1}$ .

Fig. 9 From a van Wijhe preparation of the head of an embryo 24 mm. long (W. A. C. No. 315). Right occipital and otic regions seen from the left. In this, as in all the van Wijhe preparations, the cochlear capsule is for the most part only lightly stained.  $\times \frac{1}{1.3}$ .

Fig. 10 From a model of the left otic capsule of an embryo 23.1 mm. long (H. E. C. Series 466). The large, cut surface is in a frontal plane through the middle of the vestibule. Dorsal and medial aspect,  $\times \frac{2}{1.6}$ .

Fig. 11 The same as figure 10, excepting the level of the cut surface, which is at the ventral part of the vestibule.

Fig. 12 Region of the foramen rotundum as it appears in the model of the 23.1 mm. embryo. Ventral and caudal aspect,  $\times \frac{2}{1.0}$ .

Fig. 13 Ethmoidal skeleton; from the model of the 23.1 mm. embryo. Represents the left half from its medial aspect, the septal cartilage being omitted and the nasal tectum, laminae transversales and lamina infracribrosa sectioned just to the left of and parallel with the median plane.  $\times \frac{2}{1.3}$ .

Fig. 14 From a model of the basal plate and epistropheus in median section showing the course of the notochord. Embryo 23.1 mm. long.  $\times \frac{2}{1.0}$ .

Fig. 15 Transverse section through the occipital region. Embryo 10.6 mm. long (H. E. C. Series 476, sect. 300).  $\times \frac{1}{1.5}$ .

Fig. 16 Frontal section through base of orbito-temporal region. Embryo 12 mm. long (H. E. C. Series 403, sect's. 200-207).  $\times \frac{1}{1}^5$ .

Fig. 17 Median sagittal section through base of cranium. Embryo 12 mm. long (H. E. C. Series 400).  $\times \frac{2}{1}^0$ .

Fig. 18 Median sagittal section through base of cranium. Embryo 15 mm. long (H. E. C. Series 437, sect's. 158, 161, 169).  $\times \frac{2}{1}^0$ .

Fig. 19 Frontal section through occipital and otic regions. (Sect. 246).  $\times \frac{1}{1}^5$ .

Fig. 20 Frontal section through occipital and otic regions. (Sect. 253).  $\times \frac{1}{1}^5$ .

Fig. 21 Frontal section through the otic region. (Sect. 256).  $\times \frac{1}{1}^5$ .

Fig. 22 Frontal section through the otic region. (Sect. 273).  $\times \frac{1}{1}^5$ .

Fig. 23 Transverse section through the orbito-temporal region. (Sect. 300).  $\times \frac{1}{1}^5$ .

Fig. 24 Transverse section through the orbito-temporal region. (Sect. 339).  $\times \frac{1}{1}^5$ .

Fig. 25 Transverse section through the orbito-temporal and ethmoidal regions. (Sect. 385).  $\times \frac{1}{1}^5$ .

Fig. 26 The same as in figure 25 but further forward. (Sect. 404).  $\times \frac{1}{1}^5$ .

Fig. 27 Transverse section through the ethmoidal region. (Sect. 457).  $\times \frac{1}{1}^5$ .

Fig. 28 Transverse section through the ethmoidal region. (Sect. 489).  $\times \frac{1}{1}^5$ .

Fig. 29 Transverse section through the ethmoidal region. (Sect. 492).  $\times \frac{1}{1}^5$ .

Fig. 30 Transverse section through the ethmoidal region. (Sect. 513).  $\times \frac{1}{1}^5$ .

## ABBREVIATIONS

*VI*, abducent nerve

*XI*, accessory nerve

*Ac.fis.*, acoustic fissure

*VIII*, acoustic nerve

*Al.orb.*, ala orbitalis

*Al.temp.*, ala temporalis

*Alar.*, alar element

*Amp.a.*, ampulla anterior

*Amp.l.*, ampulla lateralis

*Amp.p.*, ampulla posterior

*Ant.pl.*, antorbital plane

*Aq.coc.*, aqueductus cochleae

*Pr.at.*, atlantal process

*At.*, atlas

*Atrl.*, atrioturbinal

*Bas.pl.*, basal plate

*Can.sem.ant.*, canalis semicircularis anterior

*Can.sem.lat.*, canalis semicircularis lateralis

*Can.sem.post.*, canalis semicircularis posterior

*Car.for.*, carotid foramen

*Cav.epipt.*, cavum epiptericum

*Cav.coc.*, cavum cochleae

*Cav.nas.*, cavum nasi

*Cav.vest.ant.*, cavum vestibulare anterius

*Cav.vest.post.*, cavum vestibulare posterius

*Cent.at.*, centrum of atlas

*Cent.ep.*, centrum of epistropheus

*Ch.tym.*, chorda tympani

*Co.cap.*, cochlear capsule

*Co.d.*, cochlear duct

*Com.alic.*, commissura aliochlearis

*Com.bas.*, commissura basicochlearis

*Com.basiv.*, commissura basivestibularis

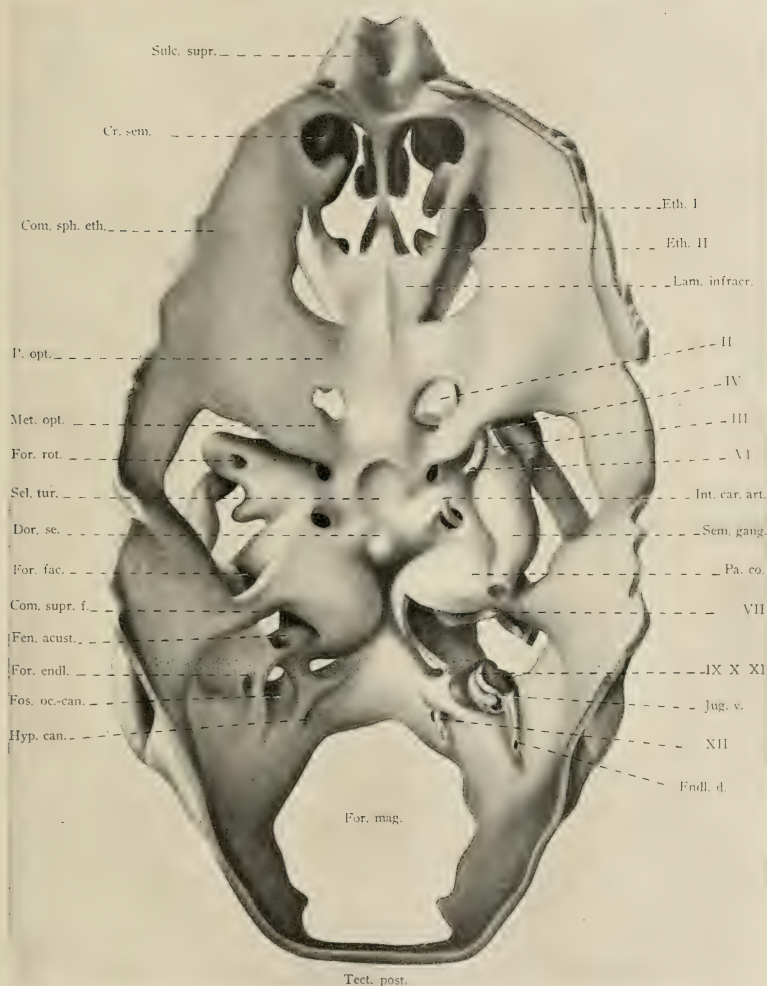
*Com.el.*, commissural element

*Com.orb.-par.*, commissura orbito-parietalis  
*Com.p.-cap.*, commissura parieto-capularis  
*Com.sph.-eth.*, commissura sphenomoidalis  
*Com.supr.f.*, commissura suprafacialis  
*Cr.int.orb.*, crista interorbitalis  
*Cr.intervest.*, crista intervestibularis  
*Cr.par.*, crista parotica  
*Cr.sem.*, crista semicircularis  
*Cr.trans.*, crista transversa  
*Den.*, dens epistrophei  
*Dent.*, dentale  
*Dor.se.*, dorsum sellae  
*D.co.*, ductus cochlearis  
*D.sem.a.*, ductus semicircularis anterior  
*D.sem.l.*, ductus semicircularis lateralis  
*D.sem.p.*, ductus semicircularis posterior  
*Endl.d.*, endolymphatic duct  
*Ep.*, epistropheus  
*Eth. I.*, ethmoturbinal I  
*Eth. II.*, ethmoturbinal II  
*Ext.meat.*, external auditory meatus  
*Oc.*, eyeball  
*VII.*, facial nerve  
*Fen.acust.*, fenestra acustica  
*Fen.bas.*, fenestra basalis  
*Fen.bas.post.*, fenestra basieranialis posterior  
*Fen.nar.*, fenestra narina  
*Fen.olf.*, fenestra olfactoria  
*Fen.sphen.-par.*, fenestra sphenoparietalis  
*Fen.vest.*, fenestra vestibuli  
*Fis.alicoch.*, fissura alicochlearis  
*Fis.bas.post.*, fissura basicochlearis posterior  
*Fis.orb.-eth.*, fissura orbito-ethmoidalis  
*For.co.*, foramen cochleae  
*For.endl.*, foramen endolymphaticum  
*For.epiph.*, foramen epiphaniale  
*For.fac.*, foramen faciale  
*For.hyp.*, foramen hypophyseos  
*For.jug.*, foramen jugulare  
*For.jug.sp.*, foramen jugulare spurium  
*For.mag.*, foramen occipitale magnum  
*For.peril.*, foramen perilymphaticum  
*For.rot.*, foramen rotundum

*Fos.inc.*, fossa incudis  
*Fos.oc-can.*, fossa occipito-canalicularis  
*Front.*, frontal bone  
*Gen.gang.*, geniculate ganglion  
*IX.*, glossopharyngeal nerve  
*Gr.sup.*, great superficial petrosal nerve  
*Hi.sem.*, hiatus semilunaris  
*Hyp.ar.at.*, hypochordal arch of atlas  
*Hyp.can.*, hypoglossal canal  
*XII.*, hypoglossal nerve  
*Hyph.can.*, hypophyseal canal  
*Hyph.cart.*, hypophyseal cartilage  
*Hyph.*, hypophysis  
*Inciv.*, incisive bone  
*Inciv.d.*, incisive duct  
*Inc.lac.*, incisura lacera  
*Inc.lacr.*, incisura lacrimalis  
*Inc.max.atr.*, incisura maxillo-atrioturbinalis  
*Inc.occ.ant.*, incisura occipitalis anterior  
*Inc.occ.post.*, incisura occipitalis posterior  
*Inc.oval.*, incisura ovalis  
*Inc.posttr.*, incisura postransversalis  
*Inc.sing.*, incisura singularis  
*Inc.cart.*, incudal cartilage  
*Inf.ac.for.*, inferior acoustic foramen  
*Inf.obl.m.*, inferior oblique muscle  
*Infr.n.*, infraorbital nerve  
*Int.car.art.*, internal carotid artery  
*J.O.*, Jacobson's organ  
*Jug.v.*, jugular vein  
*Lam.ant.*, lamina antorbitalis  
*Lam.asc.*, lamina ascendens  
*Lam.infracr.*, lamina infraorbitalis  
*Lam.par.*, lamina parietalis  
*Lam.suprch.*, lamina supraconchalis  
*Lam.trans.ant.*, lamina transversalis anterior  
*Lam.trans.post.*, lamina transversalis posterior  
*Lat.m.at.*, lateral mass of atlas  
*Lat.nas.gl.*, lateral nasal glands  
*Lat.nas.n.*, lateral nasal nerve  
*Lat.occ.*, lateral occipital arch  
*Mand.cond.*, mandibular condensation of ala temporalis  
*Mand.n.*, mandibular nerve

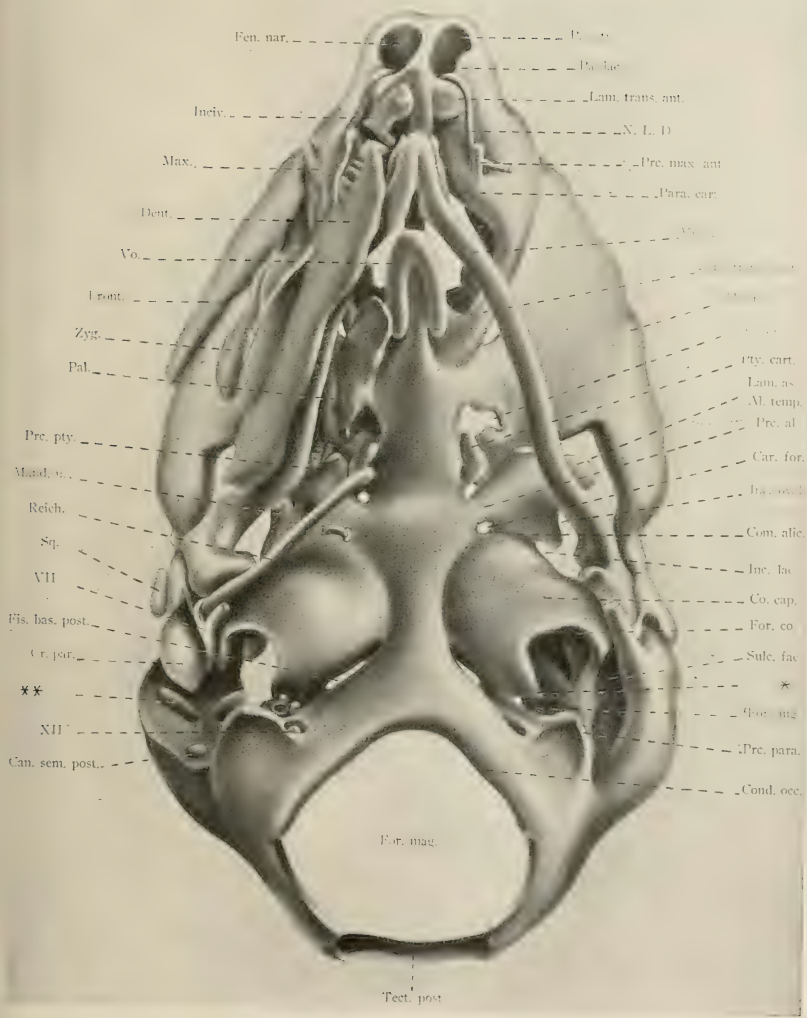


- M.*, massa angularis  
*Mast.*, masseter muscle  
*Max.*, maxillary bone  
*Max.cond.*, maxillary condensation of ala temporalis  
*Max.n.*, maxillary nerve  
*Max.turb.*, maxilloturbinal  
*Meat.ac.int.*, meatus acusticus internus  
*Meat.inf.*, meatus inferior of the nose  
*Meat.s.*, meatus supraconchalis  
*Meck.*, Meckel's cartilage  
*Memb.lim.*, membrana limitans  
*Met.opt.*, metoptic root  
*Mo.*, mouth  
*N.L.D.*, naso-lacrimal duct  
*Nas.ph.d.*, nasopharyngeal duct  
*Nasturb.*, nasoturbinal  
*Neu.ar.at.*, neural arch of atlas  
*Neu.ar.ep.*, neural arch of epistropheus  
*Noto.*, notochord  
*Cond.occ.*, occipital condyles  
*III*, oculo-motor nerve  
*Olf.*, olfactory nerve  
*Oph.*, ophthalmic nerve  
*Op.for.*, optic foramen  
*II*, optic nerve  
*Pal.*, palate bone  
*Pal.max.*, palate process of maxilla  
*Para.pl.*, parachordal plate  
*Parna.cart.*, paranasal cartilage  
*Para.cart.*, paraseptal cartilage  
*Par.nas.*, paries nasi  
*Par.-tect.*, parieto-tectal cartilage  
*Pa.atr.*, pars atrialis  
*Pa.can.*, pars canalicularis  
*Pa.co.*, pars cochlearis  
*Pa.lac.*, pars lacrimalis  
*Phary.pch.*, pharyngeal pouch I  
*Pl.suprch.*, planum supracochleare  
*Post.cup.*, posterior cupola  
*P.opt.*, preoptic root  
*Prc.al.*, processus alaris  
*Prc.max.ant.*, processus maxillaris anterior  
*Prc.orb.*, processus orbitalis  
*Prc.para.*, processus paracondyloideus  
*Prc.pt.*, processus pterygoideus  
*Prm.lat.*, prominentia lateralis  
*Prm.utr.*, prominentia utricularis  
*Prm.ut.inf.*, prominentia utriculo-ampullaris inferior  
*Prm.ut.sup.*, prominentia utriculo-ampullaris superior  
*Pty.cart.*, pterygoid cartilage  
*Pty.n.*, pterygoid nerve  
*Pty.m.*, pterygoid muscle  
*Rec.amp.ant.*, recessus ampullaris anterior  
*Rec.amp.post.*, recessus ampullaris posterior  
*Rec.lat.*, recessus lateralis  
*Rec.lat.inf.*, recessus lateralis inferior  
*Rec.lat.sup.*, recessus lateralis superior  
*Reich.*, Reichert's cartilage  
*Sac.*, sacculus  
*Sel.tur.*, sella turcica  
*Sem.gang.*, semilunar ganglion  
*Sept.*, septal cartilage  
*Sep.sp.*, septum spirale  
*Sept.tr.*, septum transversum  
*Sph.-orb.fis.*, sphenio-orbital fissure  
*Sph.p.gang.*, sphenopalatine ganglion  
*Sp.N.I*, Spinal Nerve I  
*Sp.N.II*, Spinal Nerve II  
*Sq.*, squamosal  
*St.*, stapedial cartilage  
*Stp.m.*, stapedius muscle  
*Sulc.ch.*, sulcus chiasmaticus  
*Sulc.fac.*, sulcus facialis  
*Sulc.lat.ant.*, sulcus lateralis anterior  
*Sulc.lat.post.*, sulcus lateralis posterior  
*Sulc.sig.*, sulcus sigmoideus  
*Sulc.sp.*, sulcus spiralis  
*Sulc.suprach.*, sulcus supraconchalis  
*Sulc.supr.*, sulcus suprasetalis  
*Sup.ac.for.*, superior acoustic foramen  
*Tect.nas.*, tectum nasi  
*Tect.post.*, tectum posterius  
*Temp.m.*, temporal muscle  
*Trab.*, trabecular plate  
*V*, trigeminal nerve  
*IV*, trochlear nerve  
*Tub.eph.*, tuberculum ephippii  
*Tub.jug.*, tuberculum jugulare  
*X*, vagus  
*Vert.a.*, vertebral artery  
*Vo.*, vomer  
*Utr.*, utriculus  
*Zyg.*, zygomatic bone



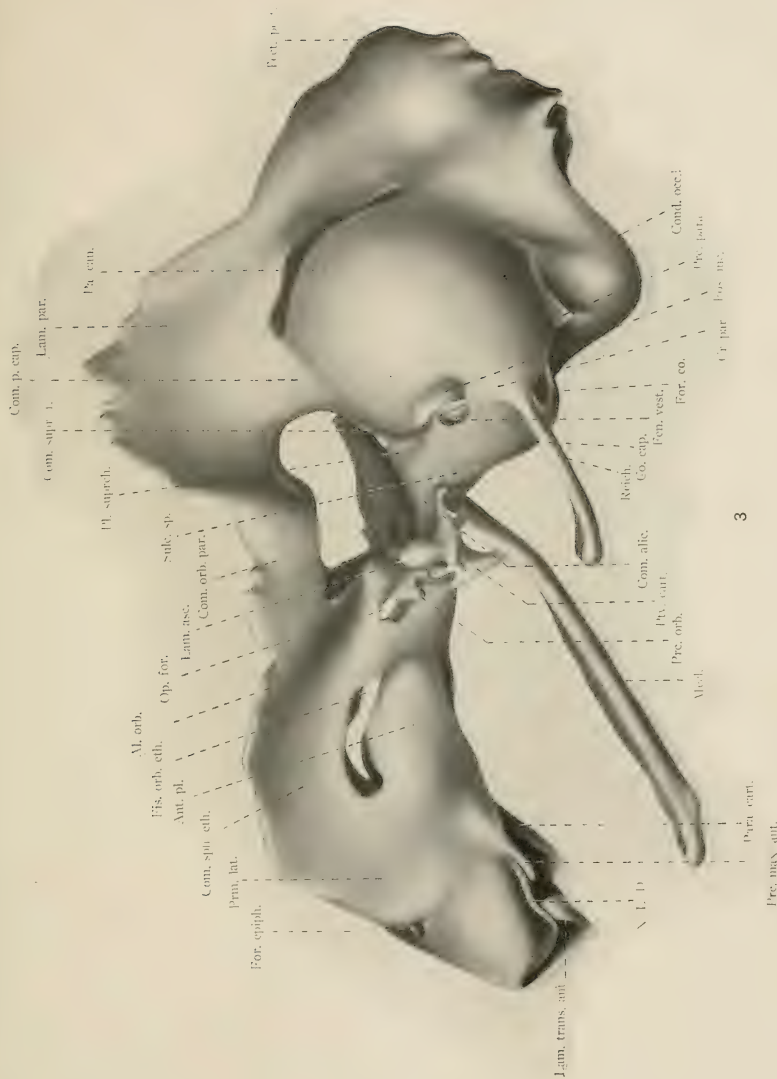
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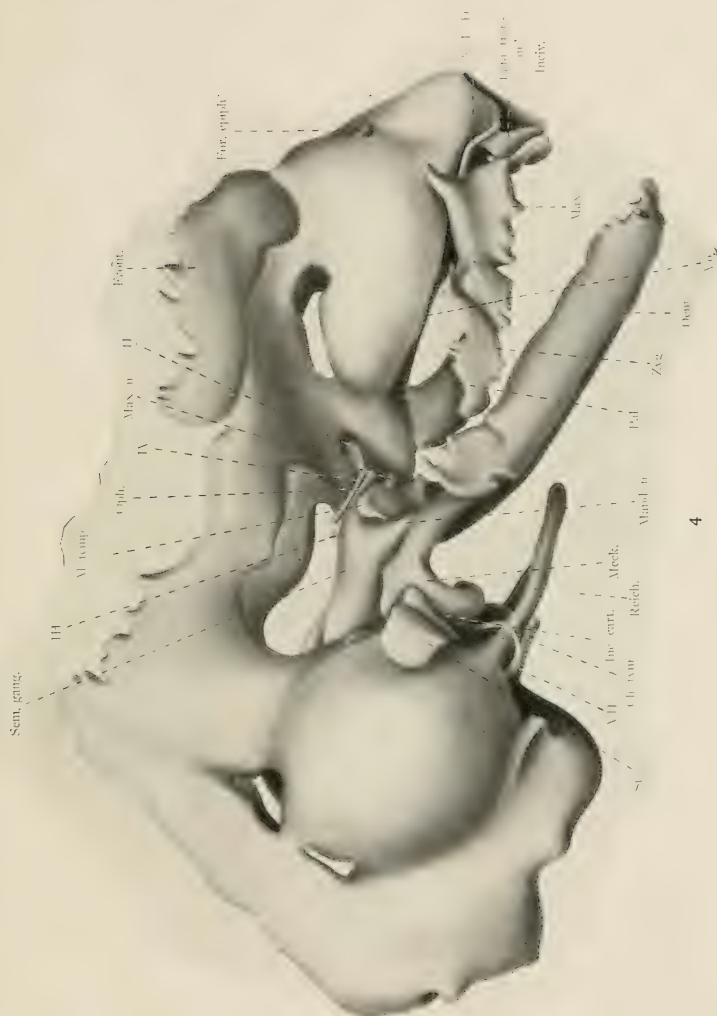










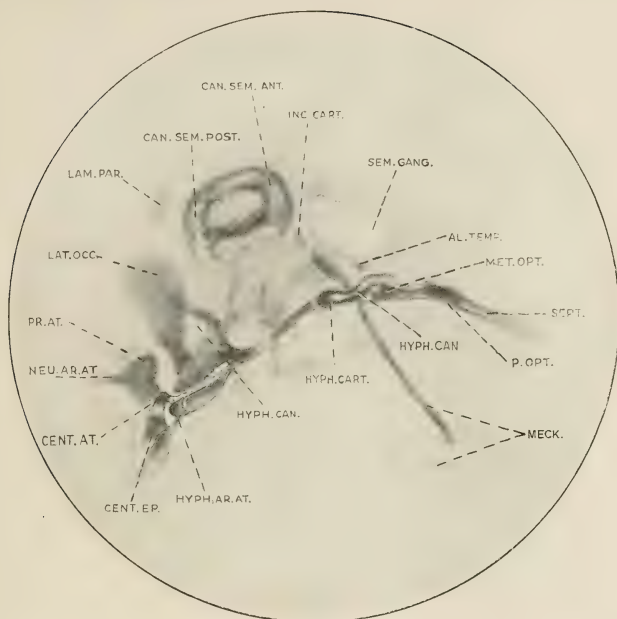




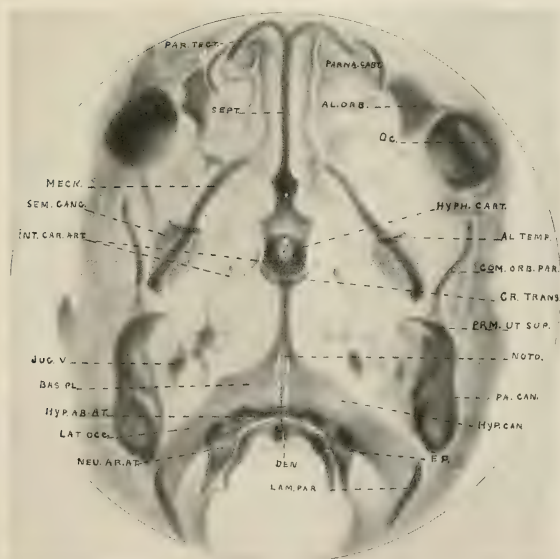






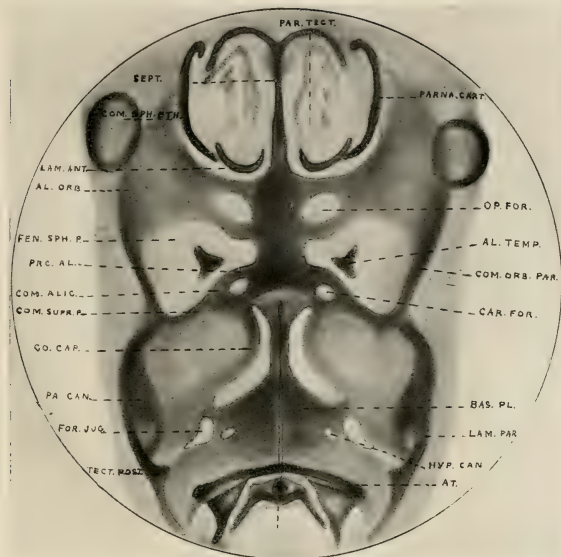


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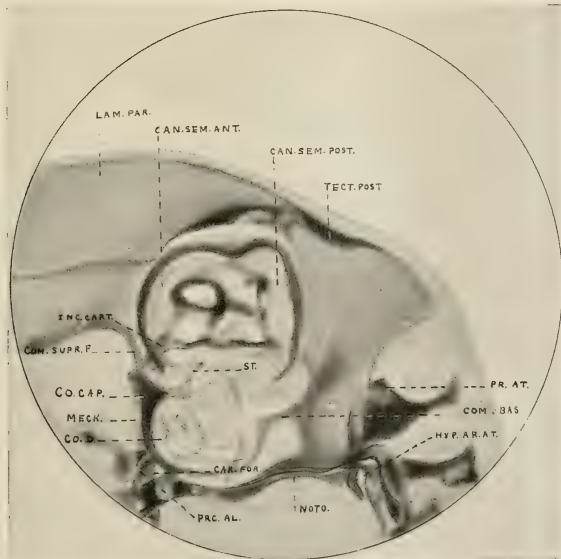


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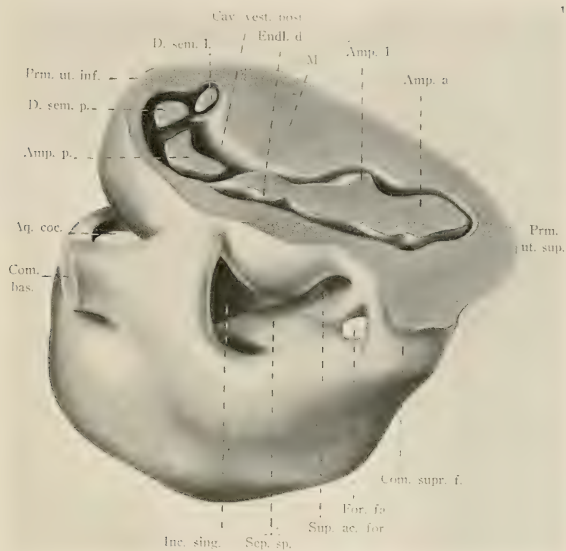
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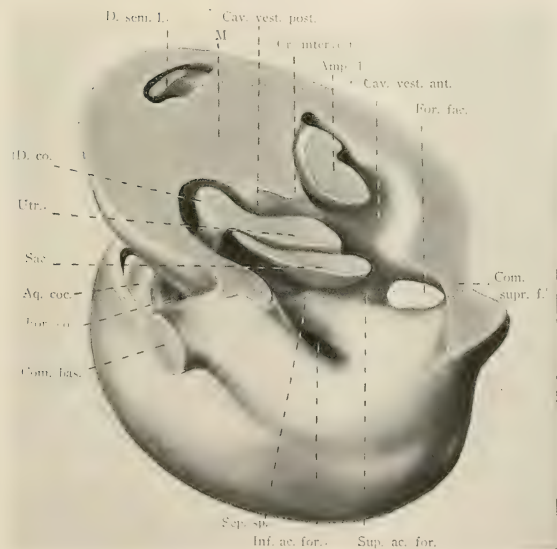
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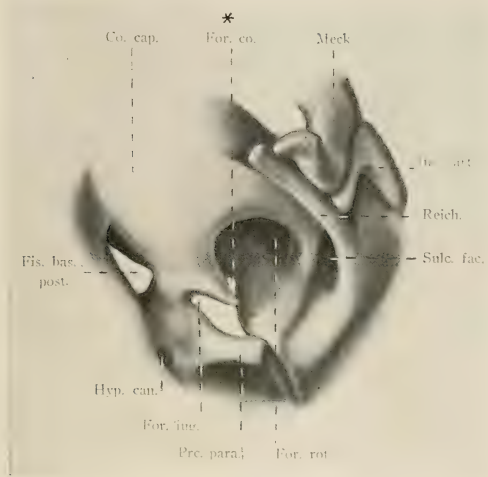


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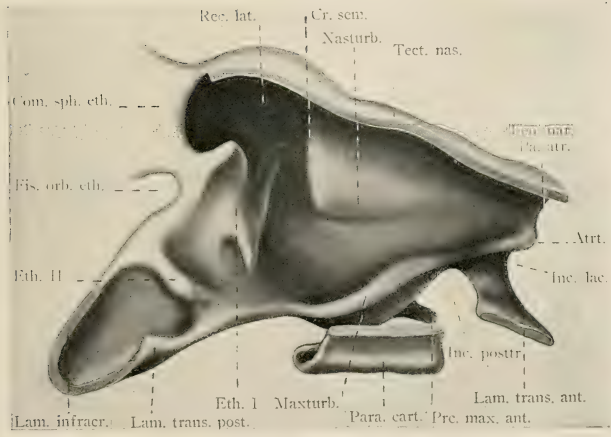


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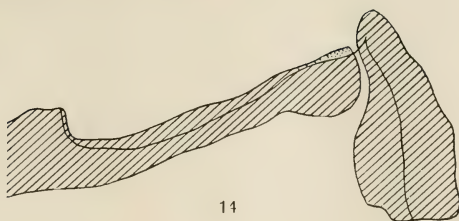
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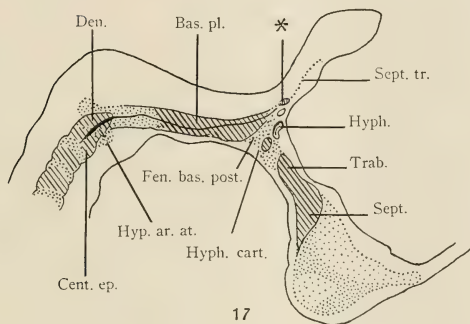
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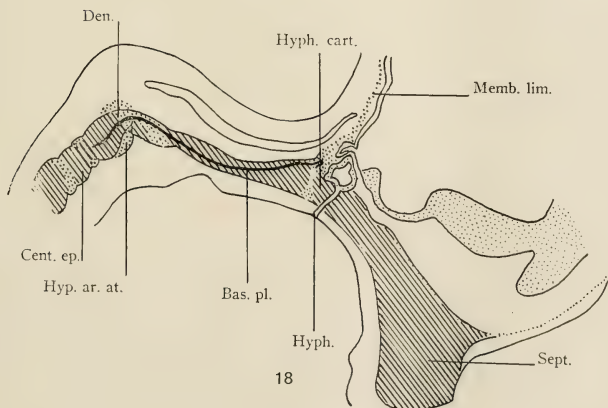




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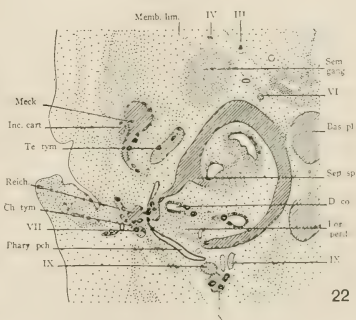
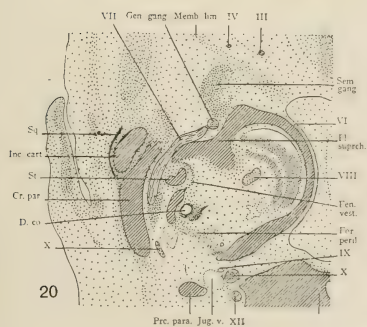
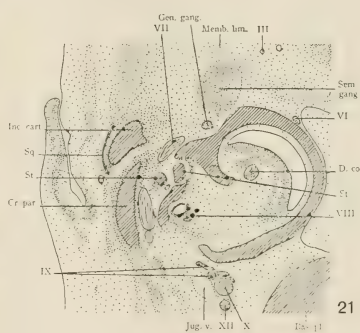
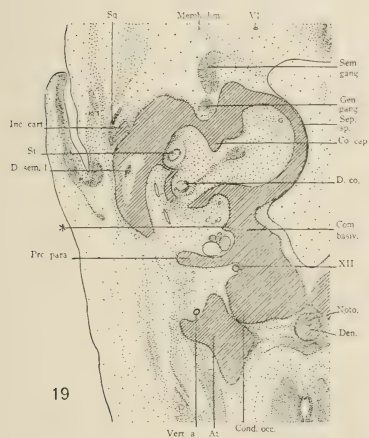
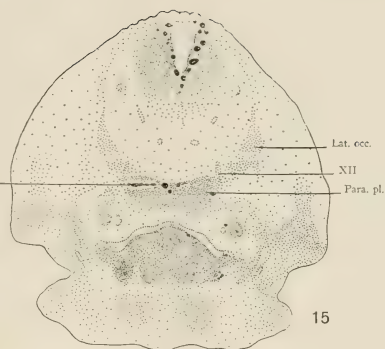
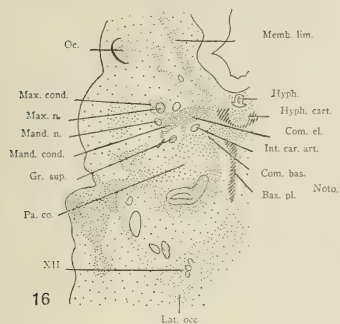
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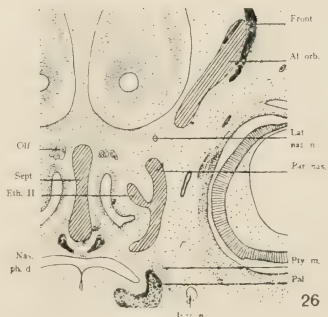
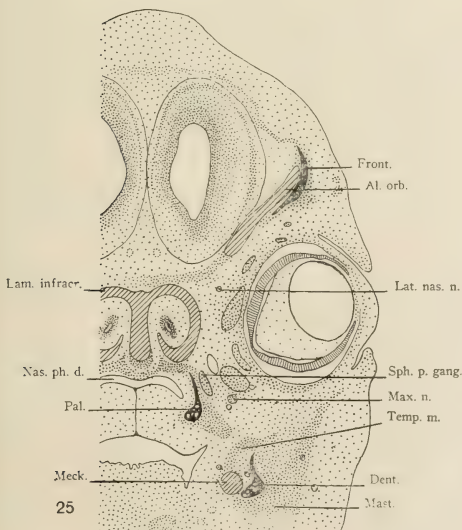
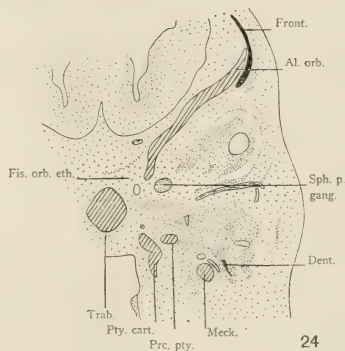
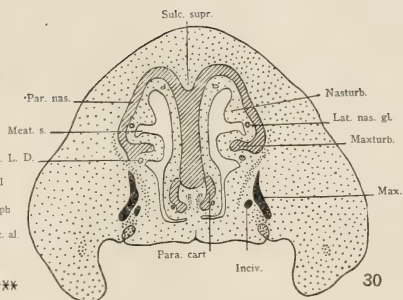
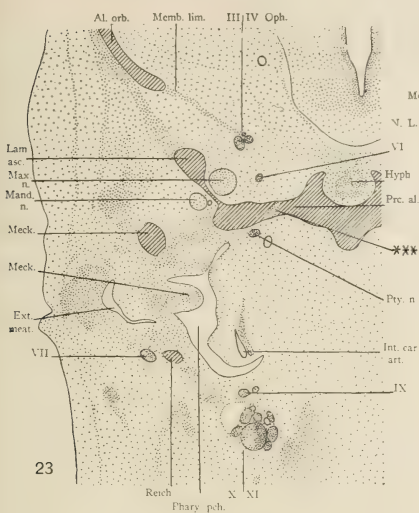
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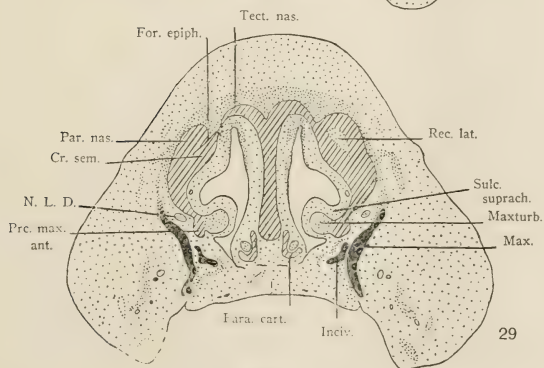
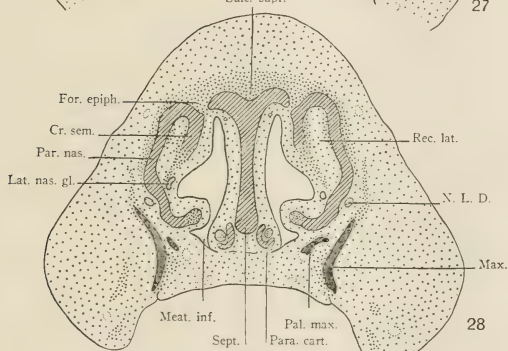
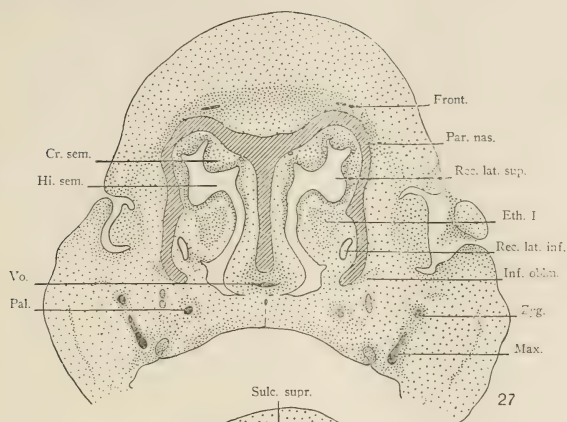














## NOTE ON THE STRUCTURE OF THE MAXILLARY GLAND OF CYPRIDINA HILGENDORFII

NAOHIDE YATSU

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FOUR FIGURES

While at Misaki in the summer of 1900 I undertook, at the suggestion of Professor Watase, a study on the ostracods with especial reference to the cytology of the luminous glands. In the winter of 1900-1901 quite a number of specimens were cut into sections and several sketches were made. But my going to America obliged me to suspend the work. Last summer ('16) Dr. Harvey of Princeton University came over to Japan and carried out at the Misaki Biological Station biochemical experiments upon the luminous substance formed in the maxillary gland of *Cypridina hilgendorffii*. In connection with his study it may be of some interest to publish the results of my observation on the morphology of the gland carried out sixteen years ago.

In *Cypridina hilgendorffii* (Müller '90, p. 228) the maxillary gland (Oberlippendrüse) attains a considerable development, consisting of a number of large gland cells. These cells open to the exterior on five protuberances, one unpaired anterior and two pairs of posterior ones (figs. 1 and 2). The anterior process has five or six pairs of openings. The middle pair is shorter than the anterior one and each has only three or four openings. The hindmost pair is the longest of all, projecting over the anterior wall of the mouth cavity, and each has seven or eight openings along its side. The last pair of protuberances alone is provided with fine hairs.

The maxillary gland is a group of unicellular glands, as have often been described by previous writers. The longest cells measure nearly 0.7 mm. in length and reach as far up as the

supraoesophageal ganglion. Their diameters vary naturally according to their secretory activity. The gland cells usually assume a club shape, tapering towards the openings. In this form as in other marine ostracods, the gland cells are not differentiated into the gland proper and the duct, contrary to those of fresh water forms (Bergold, '10). The secretion granules fill

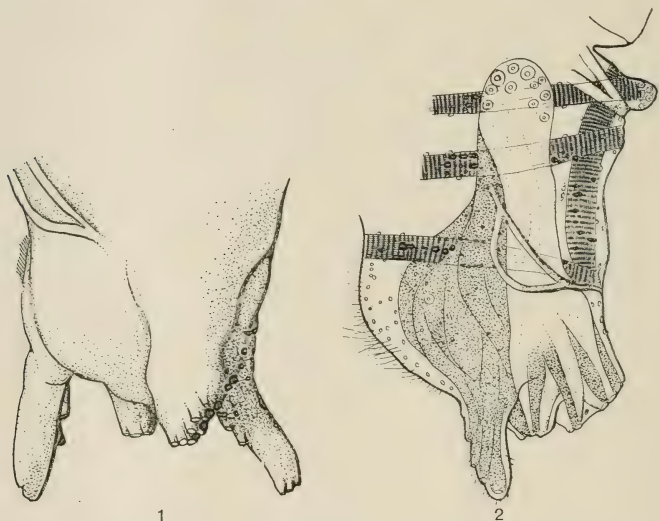


Fig. 1 Upper lip of *C. hilgendorffii* seen in an oblique view from the anterior side. One of the middle pair of protuberances is behind the anterior one.  $\times 115$ .

Fig. 2 Upper lip of *C. hilgendorffii* (♀) seen from the right side. Fresh material treated with methylgreen acidulated with acetic acid. Mucous gland cells are stippled.  $\times 90$ .

up a greater part of the cells, if specimens are killed quickly, that is, before the oozing out of the contents. But in individuals from which the secretion products have been discharged, the lower portion of the cells has a large space containing a coarse spongy coagulum. This space, especially in poorly fixed preparations, is liable to give a deceptive picture, as though there were a large reservoir common to all the gland cells.

The maxillary gland is composed of two entirely different elements, namely the mucous gland cells and the yellow gland cells. The posterior protuberances have the openings of the former alone, while both the anterior and middle ones have those of two kinds of cells (fig. 2). The secretion product of the mucous cells of the maxillary gland stains intensely green with methyl-green, as in the gland cells found in the appendages and the



Fig. 3 A portion of a vertical section through the maxillary gland of *C. hilgendorffii* (♂). Alcohol-acetic preparation.  $\times 247$ .

Fig. 4 Cross section through the anterior process of the upper lip (♀). Flemming's fluid preparation.  $\times 247$ .

mantles. In sections the cytoplasm of the mucous cells is finely granular. The other kind of gland cells I shall call the yellow gland cells, since they are yellow in life, due to the color of the secretion product they contain. The yellow substance, which emits light, is in the form of coarse, somewhat angular, granules. The size of the granules varies considerably, often attaining the diameter of 10 to 15  $\mu$ . In general it may be said, that the nearer the openings, the larger the granules. Their seat of formation is in all probability near the nucleus, inferring from the fact that a small number of minute granules of the same



nature is usually found there. The cytoplasm of the yellow gland cell is fibrillar, in contrast to that of mucous cells, which is granular as has already been mentioned. Figure 4 shows a section through the anterior protuberance near the openings. Here we see very clearly the relative position of both kinds of gland cells.

In comparison with *Cypridina hilgendorffii*, the maxillary gland of *Pyrocypis japonica* (Müller '90, p. 233<sup>1</sup>) was studied. The protuberances of the latter differ both in shape and number from those of the former, one unpaired one being added in front. But so far as the internal make-up of the glands is concerned, it is so similar that its description would be simply the repetition of what has been stated above. The only difference is the presence of pigment cells in the upper lip of *P. japonica*.

In conclusion, I would like to emphasize once more the two points, that is, the presence of two kinds of gland cells and the absence of a reservoir for secretion granules common to all the gland cells. It should be mentioned that Watanabe was the first to direct especial attention to the luminous glands of ostracods at Misaki. He read a paper on this subject before the meeting of the Tokyo Zoological Society on January 23, 1897, an abstract of which appeared in Japanese ('97). He states that the maxillary glands secrete colorless transparent fluid and yellow homogeneous substance. But he does not say whether each gland cells produces these two substances or whether there are two different glands for them. Müller ('90, p. 248) clearly states that he saw two groups of gland cells of different nature, *i.e.*, the secretion product of the upper group is found in the form of droplets and does not take carmine stain at all, while that of the lower group takes it. Furthermore he advanced the view that light is produced by the interaction of these two substances. He, however, seems to have failed to observe the correct topographical relation of these two kinds of cells.

<sup>1</sup> The general outline of the shell of *Pyrocypis japonica* is more like that of *P. chierchiai* (Müller, '90, pl. 25, fig. 3) the posterior process being longer than in the figure for this species.

One other point, which I would like to call attention, is the absence of common reservoir for gland cells. Although all the students of ostracods maintain that the maxillary glands are unicellular, yet curiously enough those who have studied the luminous glands entertain an erroneous idea that there is a special cavity to store up the secretion product. Müller ('90, p. 248), for instance, states that "die Ausführungsgänge sämtliche Drüsenzellen vereinigen sich zu einem gemeinsamen Hohlraum." Doflein published a paper on the maxillary glands of a Japanese species of ostracod, which he provisionally calls *Halocypris* (?) and gives a semidiagrammatic figure ('06, p. 134). Since pigment cells are drawn in his figure, his material may have possibly been *Pyrocypris japonica*. At any rate he interpreted the section as though there were a spacious reservoir for secretion granules. Probably influenced by Doflein's description, Lüders ('09) also mentions the presence of a special reservoir in *Gigantocypris agassizi*. It should be mentioned that the above authors seem to have studied specimens from which a greater part of the secretion products had been discharged, and it is, I think, quite natural that they have come to such an interpretation. The lower part of each gland cell functions as a temporary reservoir of the secretion granules it is true, but this cannot be called a special organ at all. As a matter of fact, as I have expressly mentioned above, there is no reservoir in the sense of previous writers.

January 9, 1917

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## THE PERIOD OF SYNAPSIS IN THE EGG OF THE WHITE RAT, *MUS NORVEGICUS ALBINUS*

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TWO TEXT FIGURES AND ONE PLATE

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### INTRODUCTION

Of the very considerable literature on synapsis much relates to vertebrates, but only a small amount to mammals. In nearly all of the publications on mammals synapsis in the male is described, the process in the ovary being investigated in only a very few cases. In view of the importance of synapsis in its relation to theories of heredity and to the behavior of the chromosomes it has seemed worth while to extend the study to the female of another mammal. Of particular interest have been the problems of the presence of an accessory chromosome, of the identity of the chromosomes throughout the series of changes, and of whether or not an actual pairing of chromosomes takes place.

<sup>1</sup> Aided in the preparation of most of the figures by the Department of Anatomy of the University of California.

The rat was selected because of its interest in connection with other studies and because of the possibility of obtaining a chronological series of embryos of known ages, and, consequently, of securing a natural series of stages.

#### MATERIAL AND METHOD

Since the period of synapsis in the egg of the rat is well along toward completion at birth, it was found necessary to obtain embryos of known age in order to complete the necessary series of slides. These were obtained in the following way. It having been clearly demonstrated that the female albino rat normally ovulates within twenty-four hours after having given birth to a litter (Long, '12), mother rats were taken from their young immediately after parturition and placed with sexually active males. Twenty-four hours later these females were taken from the males and left alone or with females only. Upon subsequent examination about 50 per cent of the rats so treated were found to be pregnant, and the age of their embryos was consequently known to within a fraction of a day. For the most part the sex organs were dissected out of these embryos before fixing, but in a few cases the entire embryo was fixed and sectioned. For those stages occurring after birth the ovaries of young of appropriate ages were used. The fixing solutions employed were Zenker's, Flemming's strong solution, Bouin's formol-picro-acetic, and sublimate acetic. The first two gave the best results and were used almost exclusively. The material was sectioned from 3 to 10 micra thick and for the most part stained in Heidenhain's iron haematoxylin. Usually a counterstain was employed, orange G or acid fuchsin, or a mixture of the two being most satisfactory. Erythrosin was used to some extent. A few slides were stained in safranin and gentian violet.

#### GENERAL CONSIDERATIONS

The process of synapsis in the rat lasts for approximately ten days, starting about seven days previous to birth and being practically completed by three days thereafter (table, p. 444). Following von Winiwarter ('00) it has been found possible to



distinguish clearly leptotene, synaptene, pachytene, diplotene, and dicty  stages, together with more or less complicated transition changes to be mentioned later. These various stages succeed each other with considerable regularity throughout the entire ovary, so that at any one time most of the germ cells are in the same stage of the synaptic process. While it is true that those nuclei least advanced in the general process of change are most likely to be found at the periphery of the ovary, there is an absence of that clear distribution of stages in concentric zones so evident in the cat, rabbit, and man (Winiwarter '00, and Winiwarter and Sainmont '08). Because of these features of the process, the difficulty of determining the sequence of stages has been reduced to a minimum.

#### THE LAST OOGONIAL DIVISION AND EARLY PRE-LEPTOTENE CHANGES

The last oogonial division occurs about seven days before birth. In the ovaries of embryos from female 389 (table, p. 444) there are a large number of division figures together with numerous nuclei which have not yet passed through the last oogonial division and also many that belong to the early stages of the oocytes. Some few division figures may still be found in embryos from female 451-6, at six days before birth. The nuclei of the oogonia in the typical resting condition are spherical. They are approximately equal to or perhaps a little smaller than the leptotene nuclei to be described later, and are supplied with a large amount of chromatin in irregular blocks. Often one or more of these blocks is comparatively large and conspicuous, somewhat suggestive of the chromatin nucleoli of the post-synaptic stages, though they never have the definiteness of outline characteristic of these later chromatin bodies. A heavy discontinuous spireme is formed during the prophase of the last oogonial division, and often the nuclei appear slightly oval at this time. The chromatin is very closely massed together during the equatorial plate stage as well as during the anaphase, so that it is extremely difficult to distinguish individual chromosomes. The last oogonial division may be distinguished from previous ones by the numerous

deeply staining granules found in the cytoplasm during the anaphase, and by the character of the chromatin in the daughter nuclei. In the very young daughter nuclei, when the nuclear wall is first appearing, the chromatin forms a somewhat flattened, closely-packed aggregation of blocks exceedingly varied in size and shape (fig. 1). No linin is evident in such nuclei. A conspicuous, very elongate, spindle-shaped remnant of the interzonal fibers and the zwischenkörper extends over the two adjoining margins of the two daughter cells containing such nuclei. These cells may be regarded as the very first stages of the primary oocytes.

*Table showing time of occurrence of synaptic changes*

| DAYS AFTER PARTURI-TION | DAYS AFTER MATING | DAYS BEFORE OR AFTER BIRTH | NUMBER OF FEMALE | CONDITION OF GERM CELLS                         |
|-------------------------|-------------------|----------------------------|------------------|---|
| 12                      | 11                | 11                         | 418              | Gonad barely distinguishable                    |
| 15                      | 14                | 8                          | 437              | Gonad well formed, multiplication division      |
| 16                      | 15                | 7                          | 389              | Last oogonial division and pre-leptotene stages |
| 17                      | 16                | 6                          | 451-6            | Pre-leptotene and contraction figures           |
| 18                      | 17                | 5                          | 577              | Contraction figures and early leptotene         |
| 20                      | 18½               | 3½                         | 454              | Leptotene and synaptene                         |
| 20                      | 19                | 3                          | 472              | Synaptene and some pachytene                    |
| 21                      | 20                | 2                          | 376              | Synaptene and pachytene                         |
| 21½                     | 20½               | 1½                         | 481              | Pachytene                                       |
| 22                      | 21                | 1                          | 446              | Pachytene                                       |
| 22½                     | 21½               | ½                          | 596              | Pachytene                                       |
| 23                      | 22                | 0                          | Young            | Pachytene                                       |
| 23½                     | 22½               | ½                          | Young            | Pachytene and diplotene                         |
| 24                      | 23                | 1                          | Young            | Pachytene and diplotene                         |
| 25                      | 24                | 2                          | Young            | Diplotene and dictyé                            |
| 26                      | 25                | 3                          | Young            | Dictyé  |

In the same ovary with these late telophase nuclei and often immediately adjacent to them are other nuclei which, for the following reasons, may be considered as directly derived from them and as constituting the early stages of the preleptotene nuclei. Although these preleptotene nuclei may lie side by side with the late telophase nuclei they are slightly larger, almost spherical and contain chromatin still in the form of numerous

blocks of irregular size and shape (figs. 2, 3, and 4) which are distributed over the inner side of the nuclear membrane. The outline of the blocks is clear cut and definite. A few linin threads may be attached to one or more of the blocks at this stage (figs. 3 and 4). As in the case of the late telophase nuclei, here, too, the preleptotene nuclei are evidently intimately associated in pairs, as is clearly proved where remnants of interzonal fibers and *zwischenkörper* persist between the cells of two 'such nuclei. It may be concluded then that the late telophase nuclei increase in size and become more spherical; and at the same time the chromatin blocks separate and become arranged about the inner surface of the nuclear membrane. In the early stages the number of these blocks of chromatin varies from twenty-two to thirty-five and seems therefore not to be significant, except in indicating a fragmentation of the original chromatin masses.

The comparatively simple condition of the contents of the nucleus represented by the peripherally arranged chromatin blocks changes gradually into an extremely complex, chromatin-linin network dispersed more or less regularly throughout the entire nucleus. The changes represented by these closely similar conditions involve a regularly progressive increase in the number of chromatin blocks, a gradual augmentation of the number and complexity of arrangement of the linin threads, and a more or less equal distribution of the chromatic material throughout the entire nucleus. (figs. 4, 5, and 6). In the earlier phases of this series of changes it may be readily seen that all of the linin threads are attached to chromatin bodies, or, that the many small, almost powdery particles of chromatin are arranged along linin threads. There is no distinct chromatin element constant in size or shape throughout this series of changes.

This whole series of changes, from the very first form of the primary oocytes to the most complex condition of the chromatin-linin network, is strongly suggestive of the migration of chromatin along linin filaments which later probably become incorporated in the leptotene threads. It is very difficult to explain in any other manner the gradual decrease in average size and the increase in number of the chromatin blocks, simultaneously with

the appearance of the linin filaments. A study of the oldest nuclei alone would hardly lead one to such an inference, but the complete series seems very convincing. Such an elaborate series of transformations intervening between the last oogonial division and the leptotene stage has not been described in the ovary of any other mammal carefully studied, though there is a slight indication of such changes in the cat (Winiwarter '00, and Winiwarter and Sainmont '08).

#### CONTRACTION FIGURES

Immediately preceding the leptotene stage, or possibly coincident with the earlier portion of it, there is a clearly defined contraction stage. This occurs about six days before birth. The chromatin-linin is eccentrically arranged in a confused mass closely packed against the nuclear wall (fig. 7). Such figures may be found throughout the entire ovary after either Zenker's or Flemming's fixation, fixation that is evidently good in all other portions of the same tissue. Since these figures are found in all portions of well fixed ovaries, and since there is no constancy whatever in regard to the direction of contraction, it is not possible to agree with Schaffner ('07), Von Hoof ('12) and others, who contend that this so-called contraction stage is an artifact due to poor fixation. It seems rather that this stage is quite as normal as those stages immediately preceding or following it. In these figures it is obvious that the eccentrically placed darkly staining mass is not of the same consistency throughout, but is composed of chromatin blocks, granules, and linin threads. Finely attenuated linin threads often extend from the chromatin knot to the opposite wall of the nuclear cavity. The whole picture strongly suggests that the complex chromatin-linin network has somehow been concentrated at one side of the nuclear cavity, and that those strands of linin attached to the opposite side of the nucleus have not entirely shared in the process.

## THE LEPTOTENE STAGE

The leptotene stage (figs. 8 and 9) which follows the contraction stage, occurs about five to three and one-half days previous to birth. It may even persist to a later date, as illustrated by figure 9, from an ovary three days before birth, in which most of the nuclei had passed over into the pachytene stage. The chromatin threads are either regular in outline with a constant size in cross-section or slightly moniliform, exceedingly fine, and very numerous. Many definite visible ends suggest that a large number of individual threads are present. As indicated in figures 8 and 9 the greater part of the threads lie at one side of the nucleus. This arrangement may well be considered as determined by the previous contracted state of the chromatin and as leading to the subsequent polarized orientation of the pachytene. In fact, the above figures show a tendency for many of the ends of threads to reach the nuclear membrane in the region near which the so-called centrosome is found when demonstrable at this stage. In figure 9 are a number of confused clumps of chromatin characteristic of early leptotene nuclei, but no definite chromatin body, uniform in either size or shape could be found at this period. A tendency toward parallel arrangement of threads is very apparent in all nuclei closely examined, and in many cases (figs. 8 and 9) figures indicating the side by side approximation of individual threads are clearly demonstrable. Where the moniliform character of the threads is appreciable, the enlargements into which the chromatin is collected tend to lie exactly opposite to each other in the more closely approximated parallel threads. These leptotene nuclei are not appreciably larger than those of the stages immediately preceding. It was not possible to determine the number of individual threads in these nuclei.

## THE SYNAPTENE STAGE

The synaptene stage may be found from three and one-half to about two days before birth. It often happens that some portions of the threads may show the bipartite nature at a much later period, but, for the most part, do not do so as late as a couple



of days before birth. The orientation of the threads first discernible in the leptotene stage is distinctly more advanced. Figure 11 is a lateral view while figure 10 is a polar view of a nucleus in the late synaptene stage. In figure 10 the nucleus is viewed from the pole opposite the so-called centrosome. It is clearly evident from figure 11 that the loops of the synaptene threads are of different lengths, some of them extending barely half as far out into the nucleus as do the others. Counts of the loops as seen in polar views or sections have not been made at this stage. Moreover, since it has not been possible to determine the number of leptotene threads, a comparison of the numbers in these two stages cannot be made as evidence of pairing. Nevertheless a consideration of the sizes and structures of the leptotene and synaptene threads give reasons for believing that the latter are derived from the former. A comparison of figures 8 and 9 with 10 and 11 makes it evident that the synaptene threads are about double the width of the leptotene. The synaptene is further clearly composed of distinct moniliform halves separated by a longitudinal split (fig. 10). Each half of the double synaptene thread is very similar to the leptotene threads, and bears much the same relation to the other half which some leptotene threads have to other leptotene threads. That is, the spindle shaped and laterally flattened enlargements in each half are usually opposite and nearly or quite in contact with those of the other half. Also the portions of each moniliform half, between the deeply staining enlargements, are almost or quite devoid of chromatin. It seems then that each of these synaptene threads was formed by the side by side approximation of two leptotene threads.

Throughout this period as in the preceding ones a chromatin body in any way simulating an accessory chromosome is not evident.

#### THE PACHYTENE STAGE

The disappearance of the longitudinal division in the synaptene thread marks its transformation into the typical thread of the pachytene stage. This stage begins two days or thereabouts before birth and persists for about a day thereafter. The ori-

entation of the previous stage is retained, though it becomes more indefinite (figs. 12, 14 and 15). The nuclei sometimes appear more oval, in contrast to the former spherical shape, and the thread is more regularly distributed throughout the entire nucleus (fig. 15), with a tendency, perhaps, toward a peripheral arrangement. The thread itself is of about the same thickness as that of the preceding stage. It is distinctly moniliform, and in early pachytene nuclei it is clear cut and definite in outline (figs. 12 and 14). The enlargements of the thread stain heavily and show no evidence of their double origin except in very early threads (fig. 12). In the intervals of the thread between these enlargements, there is a paucity of chromatin, and in some cases it seems entirely wanting, only the linin framework being left. In the later stages of the pachytene nuclei, the outline of the thread becomes less distinct due to the extension laterally of numerous very fine linin strands connecting these enlargements of the thread.

Figure 13 represents a pole view of a nucleus similar to figure 11 or 12 cut through the equator. The threads are accurately drawn as they are seen near the sphere or centrosome. They show no sign of being double and each one undoubtedly represents one end of a loop. They number 40. Consequently there would be 20 loops; and, if each loop represent paired chromosomes, the diploid number of chromosomes is 40, the haploid 20. Too great reliance, however, must not be put on the number obtained by this single count.

For the first time in this series of stages, a distinct chromatin body becomes distinguishable in the nucleus (figs. 12 and 15). This chromatin body, however, is constant neither in shape nor in size, and is often distinctly connected with one or more pachytene threads. It can hardly be regarded with reason as an accessory or sex chromosome, but is rather to be looked upon as the first appearance of the large chromatin nucleoli which characterize the nuclei of the early growth period.

## THE DIPLOTENE STAGE

Nuclei in the diplotene stage may first be distinguished at the time of birth, or possibly a little earlier, and persist in comparatively large numbers for a couple of days thereafter. At three days after birth they are but infrequently met with, practically all of the nuclei having passed over into the stage characteristic of the early period of growth and designated by Winiwarter as 'Noyoux dictyé.' The early diplotene nuclei (figs. 16 and 17) show numerous contorted threads, more or less irregular in outline, but often distinctly parallel in arrangement or wound about each other in spirals. Fine strands of linin are often distinctly visible passing from thread to thread. The number of individual threads is clearly greater than in the pachytene stage, though the exact relations in this respect could not be definitely determined. The diplotene threads are also clearly only about half as wide as those of the previous stage. The orientation, so conspicuous in the threads of synaptene and pachytene nuclei, is almost entirely lost. A large chromatin body is present in each of these nuclei, and very often an additional one or two may be discovered. The chromatin threads very commonly are united to the chromatin body in large numbers (fig. 16). Some of the threads are always connected with these bodies. It seems from an examination of a large number of these diplotene nuclei, that the individual threads are formed by a longitudinal splitting of the pachytene threads, and that the chromatin is rapidly being assembled in the large chromatin bodies. The appearance of the later phases of the diplotene stage gives further evidence of the validity of these inferences: the individual threads are no longer distinctly visible as such; often rows of granules indicate their probable former course; the linin may still be apparent, in arrangement strongly suggesting the course of the former threads. The chromatin bodies, chromatin nucleoli, are more definite in outline and typically number one to three. These later stages gradate imperceptibly into the nuclei characteristic of the early growth period.

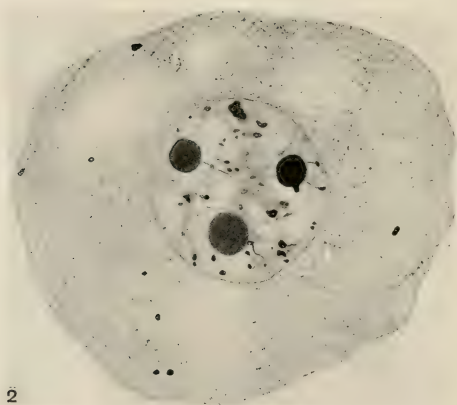
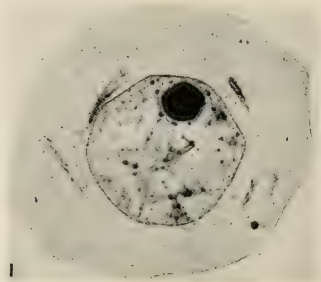
## THE NUCLEI OF THE EARLY GROWTH PERIOD

By three days after birth, most of the nuclei have passed over into the resting stage, so-called, and in this condition they remain, with certain modifications, up to the period of sexual maturity. Typically from one to three large definitely outlined chromatin nucleoli are present. Throughout the remainder of the nucleus a fine achromatic network is dispersed, with an occasional deeply staining block or particle suspended within it (text fig. 1). There is a decided increase in the size of the nucleus coincident with the beginning of the enlargement of the cell itself. The amount of achromatic material in these nuclei is strikingly greater than that of any preceding period. Its arrangement is without constant order and gives no hint of its relation to the previous stages. If it be the linin of previous stages, certainly there is here little evidence in support of that theory which postulates that the individuality of the chromosome is maintained through the constant discreteness of the linin framework.

## THE CENTROSOME AND CHROMATOID BODY

While no attempt has been made to analyze carefully the cytoplasmic changes which occur simultaneously with the series of nuclear alterations just described, our attention has been drawn to two distinct structures which appear in the cytoplasm very constantly during a part of the process. These are the so-called centrosome or body of Balbiani and the chromatoid bodies. The centrosome appears as a roughly spherical or elongated condensed portion of finer texture than the remainder of the cytoplasm, lying never more than half way from the nuclear wall to the periphery of the cell. It has no definite membrane and its outline is uneven. It is first discernible in the early leptotene nuclei (fig. 9) where it may occur in only a comparatively small percentage of the cells. Throughout the synaptene stage it is more conspicuous, and only rarely at this time is there within it a darkly staining granule, perhaps the centriole. It is very evident in most of the cells in the pachy-

tene stage, and the granule above referred to as the centriole here becomes more constantly apparent (fig. 15). At this period, in ovaries fixed in Flemming's solution, this internal granule seems to be surrounded by a narrow zone of less dense material. During the diplotene stage the centrosome can still



Text fig. 1 One of the largest cells from the ovary of a rat three days old. Fixed in Flemming's fluid and stained in iron haematoxylin.  $\times 1830$ .

Text fig. 2 One of the largest cells from the ovary of a rat nine days old. Fixed in Zenker's fluid and stained with iron haematoxylin and erythrosin.  $\times 1830$ .



be distinguished in a fairly large percentage of the cells, but it has not been found in any of the nuclei of the later stages. It will be seen that this body is evident throughout the period in the process of synapsis, when there is a distinct orientation of the chromatin threads. This orientation seems to have a definite relation to the position of the centrosome, the bent portion of the loops being, in all clear cases, directed away from that side of the cell where it occurs (fig. 12). As has already been clearly pointed out (von Winiwarter and Sainmont, '08) it cannot be asserted on the evidence here available that this body actually is the centrosome and its enclosed granule the centriole. On the other hand the converse is just as difficult of demonstration.

The chromatoid body here referred to is a deeply staining body of small size appearing, seemingly, in any region of the cytoplasm. It is rarely to be found in the cells with synaptene nuclei. During the pachytene stage (figs. 12 and 15) it is common, and here not infrequently two such bodies may be found in the same cell. From this time on throughout the period involved in this paper, it is a constant feature of a large percentage of the cells. During later stages there may be quite a number of these bodies (text fig. 2). Neither the origin nor the fate of these two structures has been determined.

#### DISCUSSION AND CONCLUSIONS

As a whole the series of changes associated with synapsis in the egg of the rat rather closely resembles the same period of development in other mammals carefully studied (von Winiwarter, '00, and von Winiwarter and Sainmont, '08). There are, however, some very noticeable differences. Those changes taking place subsequent to the last oogonial division and just previous to the formation of the leptotene threads, are much more elaborate in the rat than in any of the other forms described. In the case of the cat there is some slight suggestion in the figures (von Winiwarter and Sainmont, '08) of such a series of changes but they seem much less complex than in the rat. Particularly in the rabbit and in man there is a complete absence of those transition nuclei with the chromatin in irregular

blocks peripherally arranged, and in none of the three forms described by these authors is there that finely graded series of nuclei showing the fragmentation of these blocks and the simultaneous appearance of linin threads. In the case of the rat, the contraction stage certainly must start earlier than in these other forms. It would seem from the figures that this stage persists longer in the rabbit and in man (von Winiwarter, '00).

It has already been mentioned that distribution of nuclei in zones—the most advanced stages being in the interior and the least advanced at the periphery with the intervening stages in more or less definite concentric areas—is almost completely lacking in the case of the rat. It would seem that this phenomenon might be correlated with the comparatively shorter period over which these nuclear changes extend in this form.

In the case of the cat (von Winiwarter and Sainmont, '08) there is a clearly defined chromatin element, fairly constant in appearance, persisting throughout this entire series of changes. There is certainly no such body in the case of the rat; and von Winiwarter finds none in either the rabbit or man. In all other respects, particularly in reference to the gross as well as the more detailed appearance of the chromatin threads throughout the entire series of changes, the four forms are closely similar.

Of particular interest throughout this study has been the relation of the chromatin to the linin threads, and of the chromatin nucleoli of the later periods to the synaptene and diplotene threads. During the first part of the series of stages here described, the evidence is very strongly in favor of the conception that fragmentation of the original chromatin blocks and migration of these chromatin fragments along the linin threads then evident, contribute largely to the formation of the leptotene threads. This process is almost exactly reversed at the other end of the process of synapsis. The chromatin nucleoli seem to be merely the closely compacted clumps of that same chromatin which has now migrated back along linin threads to one or more common points. The contact of the synaptene and diplotene threads with these chromatin bodies, and the persisting linin

threads in the remainder of the nuclei, point to this conclusion. Such a phenomenon has been elaborated upon by McGill in the case of the Dragonfly (McGill, '06), and has been described by numerous other authors.

In regard to those questions which attracted attention in the first place, it seems: (1) There is no evidence, during the period of synapsis, of the presence of an accessory chromosome or sex chromosome. (2) If it be assumed that the individual leptotene threads represent chromosomes, chromosomes seem to pair during synapsis; and this is a side by side pairing, or parasynapsis. (3) Under this same assumption, it would seem that individual chromosomes maintain their identity throughout this period up to and including the diplotene stage, although there is opportunity for mixture of material in the chromatic enlargements during the pachytene stage. There is, however, no satisfactory evidence that these individual leptotene threads do represent chromosomes.

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PLATE



## PLATE 1

### EXPLANATION OF FIGURES

Figures 1, 2, 3, 7, 9, 11, 12, 16 and 17 are drawn to the same magnification ( $\times 2290$ ) with a Zeiss 2 mm. 1.4 n. a. apochromatic objective; figures 13, 14 and 15 ( $\times 2270$ ) with a Zeiss 1.8 mm. achromatic objective; and figures 5, 6, 8 and 10 ( $\times 2490$ ) with a Leitz  $\frac{1}{2}$  achromatic objective; a no. 12 Zeiss compensating ocular being used in all cases.

Figures 1 to 4, 6, 7, 9 to 11, 16 and 17 prepared by Dr. H. M. Gilkey; and figures 12, 18 and 19 by Mr. Willard Shepard.

1 to 6 Successive phases of the transition nuclei between the last oogonial division (fig. 1) and the contraction stage. From young of female 389, fixed in Zenker's fluid and stained with Heidenhain's iron haematoxylin and acid fuchsin.

7 Two nuclei in the contraction stage. Fixed in Zenker's fluid, from young of female 451-6 and stained with Heidenhain's iron haematoxylin and acid fuchsin.

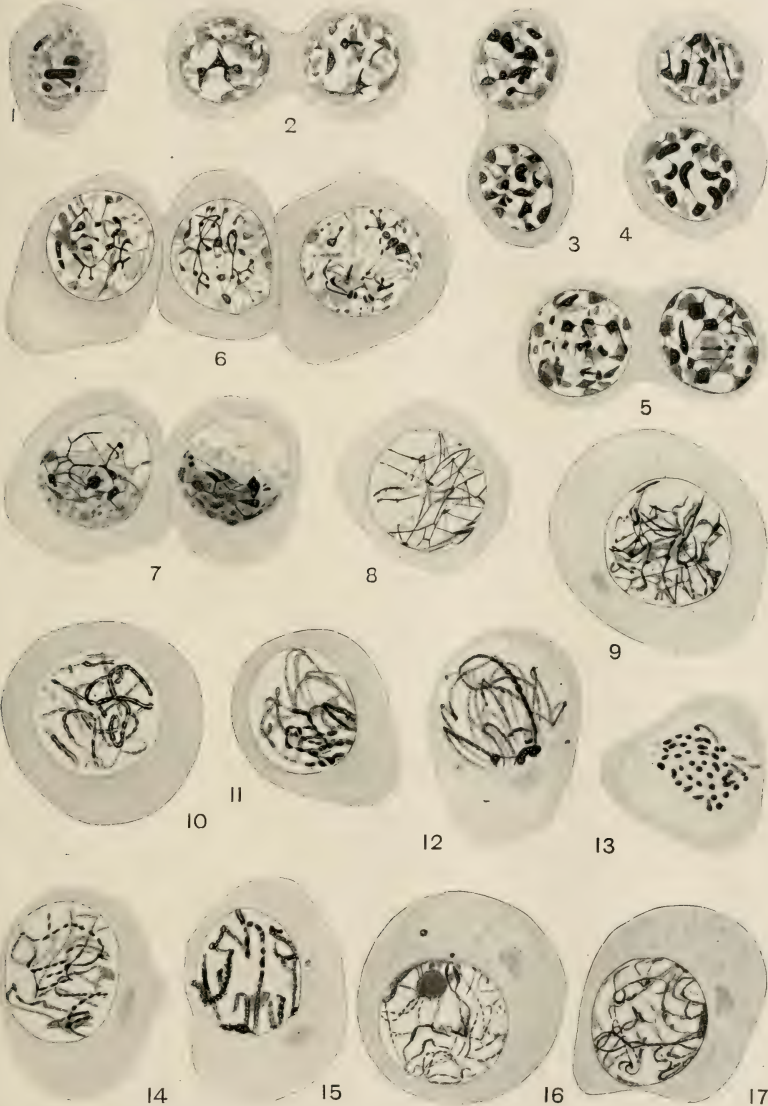
8 Side view of nucleus in the leptotene stage. Material from female 454, fixed in Zenker's fluid and stained in Heidenhain's iron haematoxylin and orange G.

9 Lateral view of a nucleus in the leptotene stage. Material from female 472, fixed in Zenker's fluid and stained with iron haematoxylin and orange G.

10 and 11 Nuclei in synaptene stage, 10 polar view, and 11 a lateral view. Material from female 376, fixed in Zenker's fluid and stained with iron haematoxylin and orange G.

12, 13, 14 and 15 Nuclei in the pachytene stage. Material of 13 from female 376, and that of 14 from female 481, both fixed in Zenker's fluid and stained in iron haematoxylin and orange G. Material of 12 and 15 from young at birth, fixed in Flemming's fluid and stained with iron haematoxylin. Figure 12, an early pachytene, and figure 14 a later, both in side view. 13 is a pole view. 15 a very late pachytene in which polarized arrangement of threads is lost.

16 and 17 Nuclei in the diplotene stage. Material from young one day old. Fixed in Zenker's fluid and stained with iron haematoxylin and orange G.





## THE EARLY DEVELOPMENT OF A STARFISH, PATERIA (ASTERINA) MINEATA

HAROLD HEATH

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### FIVE FIGURES

During the past summer a small number of gastrulae were discovered in the plankton of Monterey Bay, California, which exhibited a unique combination of characters, relating them on one hand to the Echinodermata and on the other to the Enteropneusta. To discover their true relationship all were placed in balanced aquaria where they subsequently developed into bipinnariae. Ova of various species of shore-dwelling starfishes were then artificially fertilized and reared for a sufficient length of time to show that the first captured specimens were the young of *Pateria (Asterina) mineata*.

Judging from artificially fertilized material the segmentation and early blastula stages do not exhibit any especially noteworthy features, but beyond this point certain structures arise that have no known counterpart among starfishes. The first of these unique organs is the apical plate. In the late blastula stage the cells about the animal pole commence to elongate and, in the gastrula, form a thickened area, more or less lens-shaped in form, having approximately one half the diameter of the transverse axis of the embryo. As indicated in the drawings (figs. 3, 5) its center is exactly opposite to the blastopore and therefore is strictly apical.

Sections show the component cells to possess a height fully three times that of the average ectoderm cell of the animal half of the embryo. Passing outwardly the altitude diminishes at a fairly uniform rate until the outer limits are reached. The nuclei are distally located and therefore similar in this respect

to the ectodermal elements generally. Furthermore all of the cells of the apical plate are packed with granules, evidently yolk, and thus are in marked contrast to the remaining ectoderm where the granules are relatively few and minute.

Generally speaking, this condition of affairs continues to the close of gastrulation. In specimens entering upon the initial stages of enterocoel formation, where the walls of the blind end of the archenteron have grown thin, a few of the cells of the apical plate are usually to be seen in the act of migrating into the blastocoel. As will be seen later these and other migrants from the apical plate form mesenchyme.

The formation of the enterocoels (hydroceles) proceeds in the typical fashion. In practically every case they arise independently of each other, and from the outset the left is almost invariably the larger of the two (fig. 1). Mesenchyme cells arise as usual from the walls of the enterocoels as well as from the intervening portion of the archenteric wall. Their numbers, when compared with those in *Asterias ocracea* for example, are relatively small, and, in comparison with the migrating cells of the apical plate, they are distinctly smaller and far more hyaline in appearance—features which enable one to distinguish the two types of mesenchyme (ectodermal and entodermal) throughout the stages described in this paper.

In fully 50 per cent of the specimens in hand a few of the mesenchyme cells arising from the blind end of the archenteron, between the enterocoel pouches, unite to form a small vesicle (fig. 1, *av*). This usually occurs after the enterocoel pouches are well differentiated though not completely cut off. In a relatively short space of time this anterior, unpaired vesicle is rapidly enlarged by the addition of other mesenchyme cells from the archenteron, and ultimately attains a diameter equal to that of the gut. In a few surface views it is clearly seen to be entirely enclosed, with walls everywhere complete, and, though it is closely attached to the distal wall of the archenteron, it is nevertheless distinctly separated from it. In other cases the vesicle is so closely applied to the archenteric wall that it is not possible to determine whether the vesicular walls are complete. In



every instance, however, the distal wall of the archenteron is continuous, and the evidence is perfect that this anterior vesicle is entirely formed from mesenchyme and not as an outpouching of the archenteric wall.

By the time that the anterior vesicle has attained a diameter equal to that of the archenteron the mesenchyme migration from the apical plate is at its height. Many cells in sections are seen to have become entirely free, while others are in the act of slipping past what are to become the final ectodermal elements, and are making their way into the blastocele. In several larvae they come in contact with the anterior vesicle (fig. 1), and cover it like a roof. Where the mesenchyme is more scattered, a relatively small number of cells rest upon the vesicle, while the others pass around it, especially in the region of the future oesophagus.

Beyond this stage in the development, the walls of the anterior vesicle usually disappear, the mesenchymal elements separating and migrating to other regions. In the oldest stage where the vesicle has been found to exist the stomodaeum had completely formed, and the enteroceles had severed their connection with the gut. The vesicle in question showed slight signs of disintegration on its posterior face, but, as figure 4 indicates, it is fairly complete, and has a diameter approximately equal to that of the middle section of the body. Here it is a question whether it has a complete wall next to the gut, but there is no doubt whatever about its lack of communication with the digestive tract.

Surface views under fairly high magnification show the wall of the vesicle to be made up of a relatively small number of cells. These possess highly ramified pseudopodial processes which appear to form an extremely delicate granular meshwork. Whether the meshes are bridged by a non-granular ectoplasm or by some intercellular material has not been determined, but it is evident that the bounding wall, as a whole, is fairly complete, since neither the pseudopodia of other cells nor minute granules which appear to have escaped from some of the apical cells make their way into the cavity of the vesicle. In some

specimens the blastocele contains a ground substance distinctly stained by Delafield's haematoxylin, and is to that extent in contrast with the fluid contained in the vesicle, which has only a slight affinity for dyes of this character.

After cells of the anterior vesicle have separated and migrated into the blastocele, many of the mesenchyme elements from the apical plate move to the wall of the gut, especially in the region of the oesophagus. In the oldest larvae in my possession (about twenty hours later than the stage represented in figure 2) a few of these apical mesenchyme cells, more or less bipolar in form, rest upon the wall of the anterior half of the oesophagus at right angles to its long axis. Between this point and the stomach other mesenchyme cells of entodermal origin are taking up similar positions. Both types of mesenchyme doubtless become transformed into circular muscles.

The left hydrocele, after separating from the archenteron, is not only larger than the right, but its subsequent growth is more rapid, and it soon develops a pore canal and hydropore which have no counterparts on the opposite side; at least none have been discovered in the material at hand.

A posterior enteroceles pouch (fig. 4) arises from the left side of the gut at about the middle of the future stomach. It is a hollow outgrowth, nearly as thick-walled as the gut itself, and after severing its connection with the digestive tract, it becomes a flattened vesicle wedged in between the stomach and body wall. In a stage slightly older than the one represented in figure 2 the left hydrocele forms a diverticulum at its posterior end which ultimately comes in contact with the posterior enteroceles pouch and fuses with it. Before this fusion takes place the posterior enteroceles vesicle, in several larvae, assumes the form of a rather narrow ellipse with its long axis extending from a point slightly behind the left hydrocele to the base of the intestine. Nothing is known regarding its subsequent history.

*Comparisons.* Since the year 1869, when Metschnikoff first called attention to the resemblance between the echinoderms and *Balanoglossus* a vast amount of data has accumulated. This tends to support the original theory that the two groups in ques-

tion are genetically related, but the interpretation of certain details is not completely satisfactory. For example there is a lack of agreement relating to the homologies of the coelomic cavities. It will be remembered that *Balanoglossus* develops five pouches, an anterior unpaired vesicle and two succeeding pairs which become the proboscis, collar and trunk coelom respectively. In *Balanoglossus clavigerus*, according to Heider ('09) the anterior vesicle arises as a diverticulum from the distal end of the archenteron, and after separating completely, forms the future proboscis pore, before coming in contact with the apical plate. After a time the vesicle withdraws from the plate, though retaining its connection by means of a muscular strand, while mesenchyme cells from the strand and undetermined neighboring regions migrate to the oesophageal region where they become transformed into circular muscles.

The resemblance in the behavior of the anterior vesicle in *Balanoglossus clavigerus* and *Pateria mineata* is decidedly striking. In the starfish the vesicle arises in the form of mesenchyme cells which subsequently unite; in *Balanoglossus* it originates as a direct outgrowth of the archenteron. In *Asterias rubens* and a few other species of starfishes Gemmill (14) has described the rudiments of posterior enterocele pouches which either fuse with the left hydrocele or break up into mesenchyme (and possibly unite later with the hydrocele, though this is not indicated). In other words it is well known from this and numerous other instances that there is no fundamental difference between a vesicle formed as an outpouching of the gut and the precocious development of mesenchyme with subsequent fusion. In *Pateria mineata* the anterior vesicle soon disappears, the component mesenchyme cells wandering off into the blastocele, but before its disappearance it has come into close contact with wandering cells from the apical plate. No pore unites this anterior vesicle with the exterior, but its behavior is such that I am convinced it is the homologue of the proboscis coelom of the Enteropneusta.

There is, so far as I know, no other starfish which develops a distinct, independent anterior vesicle. In several species the blind end of the archenteron becomes expanded, thin-walled and

develops laterally the two well known enteroceles, so that the median section between the pouches may possibly be considered an incipient unpaired vesicle as certain authors have suggested. On the other hand it is equally possible that some of the mesenchyme cells migrating from the distal end of the archenteron represent the anterior vesicle though they may never actually fuse. In *Pateria mineata*, for example, not over 50 per cent of the embryos form a complete vesicle; in a few cases, relatively, it is imperfect, and it appears probable that in the others it is represented by isolated mesenchyme cells which arise in the proper position but never unite. Furthermore, it is a significant fact that in all of these cases, even where the anterior vesicle is perfectly formed, the enterocoele pouches are separated by a thin section of archenteric wall which therefore can scarcely be regarded as representing the anterior vesicle.

Gemmill's discovery of a pair of posterior enterocoele pouches in the starfish is of the highest importance. They have the same origin as the trunk coelom in *Balanoglossus*, and as Gemmill states "We have here, I think, rudiments of a paired posterior enterocoele outgrowth, which in the common ancestor of *Balanoglossus* and the Echinoderms gave rise to the coelom of the body or trunk." In some of the species studied by Gemmill "this rudiment takes no part, or only a small part, in the formation of the wall of the posterior coeloms, but it still retains the function of producing mesenchyme." In *Pateria mineata*, on the other hand, it is a relatively large vesicle, present on the left side only, and in the future an attempt will be made to determine its fate. It is possible that, in this species and in the others where it forms mesenchyme, this posterior outgrowth does play a more or less important part in the history of the left enterocoele from which the hydrocele arises, and that some of the difficulties in homologizing this last named cavity are due to its compound character.

To sum up: I am strongly inclined to look upon the anterior vesicle in *Pateria mineata* as the homologue of the proboscis coelom of *Balanoglossus*, while the posterior outgrowth corresponds to the trunk coelom, and the intermediate pair of vesicles



in the echinoderms, often with two hydropores in certain species, is the equivalent of the collar coelom.

A word remains to be said regarding the apical plate. In certain crinoids and echinoids it becomes a well developed organ in fairly old larvae, in which definite ganglion cells appear. As the adult nervous system arises from the ectoderm in close proximity to the hydrocele, it would appear that these embryonic nerve cells play no part in the process. Whether they degenerate or become mesenchyme remains undetermined. It is fairly certain, however, that they furnish a strong bit of evidence in favor of the theory that the apical plate of the trochophore larva and of the echinoderm larva are homologous structures.

In *Pateria mineata* it has been shown that many of the cells of the apical plate completely lose their connection with the ectoderm and, as mesenchyme, migrate through the blastocele to the oesophageal section of the gut where they probably become transformed into circular muscles. All signs that they ever functioned as nervous elements appear to be wholly lost. In this connection one calls to mind the work of Katschenko ('88), Platt ('94) and others who find that numerous cells leave the neural crest in the head region of various vertebrate embryos and become mesenchyme. It is possible that such cells represent ganglionic elements in the primitive ancestor which formerly innervated preotic structures now vanished or rudimentary. The matter, however, remains unsettled for the vertebrates; such is emphatically the case with the echinoderms where the species studied are few indeed and the data far too scanty to enable one to draw trustworthy conclusions.

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## PLATE 1

### EXPLANATION OF FIGURES

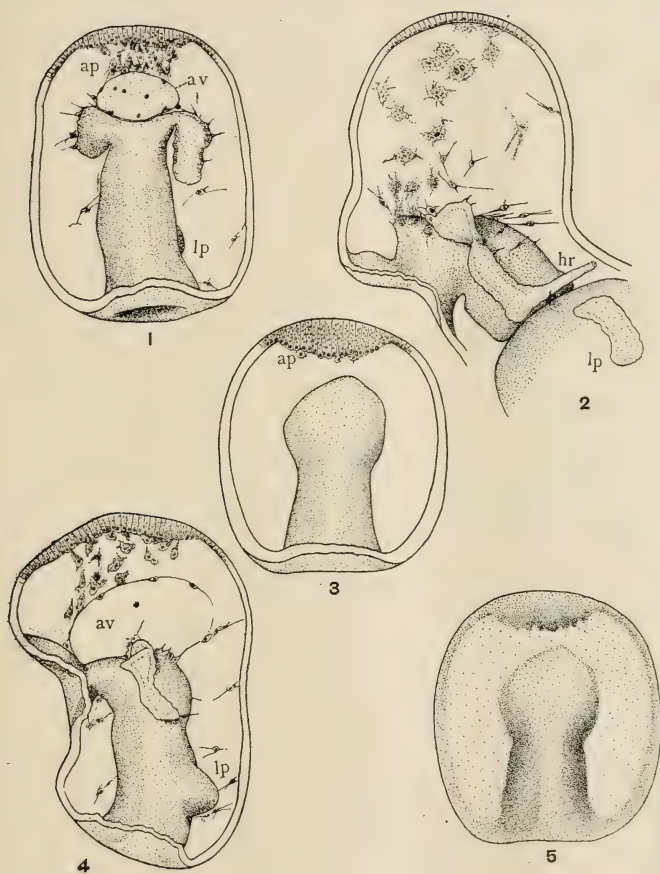
1 Late gastrula or early bipinnaria stage showing mesenchyme migrating from the apical plate (*ap*) and coming in contact with the anterior vesicle (*av*). The archenteron bears the usual two enterocoele pouches and a single left posterior one.

2 Anterior end of bipinnaria showing the completion of the mesenchyme formation at the apical pole and its migration to the esophageal region. The left hydrocele is provided with pore canal and hydropore. *lp* left posterior vesicle.

3 Section of gastrula showing apical plate with signs of mesenchyme formation.

4 Bipinnaria showing mesenchyme migrating past the anterior vesicle (*av*) to the esophagus. The left hydrocele has severed its connection with the archenteron. *lp* left posterior vesicle in process of formation.

5 Surface view of gastrula immediately before mesenchyme development from apical plate.





# SYNOPSIS AND CHROMOSOME ORGANIZATION IN CHORTHIPPUS (STENOBOTHRUS) CURTI- PENNIS AND TRIMEROTROPIS SUFFUSA (ORTHOPTERA)

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THREE PLATES

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## I. INTRODUCTION

In a recent paper (Wenrich, '16) the writer presented what seemed to him conclusive evidence that parasynapsis occurs in at least three pairs of first spermatocyte chromosomes of *Phrynotettix magnus*. Although only these three chromosomes were studied in great detail, sufficient attention was given the other members of the complex to assure the writer that parasynapsis occurs in all. *Phrynotettix*, however, like many of the *Oedipodinae*, possesses only rod-shaped, or telomitic chromosomes. It seemed advisable, therefore, to investigate the conditions to be found in species of grasshoppers in which the chromosomes are V-shaped, or atelomitic. *Chorthippus* (*Stenobothrus*) *curtipennis* was chosen for this study because it is readily obtainable, gives clear figures when fixed and stained, and because various species of the genus have been the subject of investigation in the past. Sufficient study of this species, which has three pairs of atelomitic chromosomes, was made before the publication of the paper on *Phrynotettix* to assure the writer that the chromosome relationships in synapsis are essentially the same as in that form with respect to both types of chromosomes. This is indicated by the following statement (Wenrich, '16, p. 98):

I have recently made a study of the conditions in *Stenobothrus* and may say that I found parasynapsis for both forms of chromosomes, and that the V-shaped chromosomes divide reductionally in the first maturation mitosis as Davis ('08) described, but that the rod-shaped chromosomes divide equationally in the first division as I found that they did in *Phrynotettix*.

No drawings were presented to support these statements at the time, but the evidence (with additions) on which the conclusions as to parasynapsis were based is presented in the present paper. Unfortunately, more careful study of the material makes it impossible to be so confident as to which division is reductional for the two types of chromosomes. This point will be discussed later.

The same conclusions as to parasynapsis in *Chorthippus*, together with the same kind of evidence, in part, has recently



been presented by Robertson ('16). In many respects the evidence and conclusions which have been independently reached by Robertson and myself will be mutually corroborative. In regard to some points, however, differences of interpretation exist which will be discussed in an appropriate place.

In view of the very interesting conditions in *Trimerotropis* and *Circotettix*, where telomitic chromosomes are paired with atelomitic ones, as found by Dr. E. Eleanor Carothers ('17) it seemed worth while to examine stages which would indicate what form of synapsis occurs between these chromosomes of diverse form. Dr. Carothers very kindly permitted the use of her slides for this purpose. It may be stated at once that parasynapsis was found to occur for all the chromosomes of *Trimerotropis* of whatever form, just as it does in *Chorthippus* and *Phrynotettix*. In addition to the subject of synapsis the topics of pre- and post-reduction and chromosome organization will be considered.

In this paper new terms will be used in accordance with those recently adopted by Dr. Carothers ('17), as follows: (1) *Telomitic*—chromosome with terminal fiber-attachment, i.e., rod-shaped; telomitic and rod-shaped will therefore be used interchangeably; (2) *atelomitic*—chromosome with non-terminal fiber-attachment = V-shaped; V-shaped and atelomitic will be used interchangeably; (3) *heteromorphic*—those pairs of chromosomes (tetrads) of which one member is telomitic and the other atelomitic, or, of which one member is noticeably different in form or size from its mate; (4) *chromomere-vesicle*—the plasmosome-like appendages formerly called 'vesicles' by Carothers ('13), and called appendages and plasmosome-like structures by the writer ('16). I shall also make use of McClung's ('14) term, *euchromosome* as equivalent to Montgomery's 'autosome.'

All drawings have been outlined with a pencil under the camera lucida and then inked while the cell remained under observation.

## II. OBSERVATIONS

*A. Chorthippus (Stenobothrus) curtipennis*

*a. Introductory statement.* *Chorthippus (Stenobothrus) curtipennis* belongs to the sub-family Truxalinae of the Orthopteran family Acrididae. The material from which nearly all the drawings were made consists of a single smear prepared during the summer of 1914 at Woods Hole, Mass., near which place this species was abundant. Through the kindness of Professor McClung I was afforded facilities for preparing and staining cytological material at that time. The smear was fixed in Flemming's stronger solution (at a temperature of about 4°C.). One part was stained with Zwaardemaker's safranin, the other with Heidenhain's haematoxylin. Figures 12, 13, and 14, plate 3, are from sectioned material, fixed in cold Flemming's fluid and stained with Heidenhain's haematoxylin.

While this material was studied some during the summer of 1914 and at Harvard University during the winter of 1914-1915, it was carefully re-examined in 1915-1916 at the University of Pennsylvania and again at Woods Hole during the summer of 1916, when most of the drawings were made. I am indebted to the University of Pennsylvania and to the director of the Marine Biological Laboratory for facilities for pursuing this study at Woods Hole.

Although material from a number of individuals was studied, nearly all the drawings are made from a single smear derived from one animal as stated above. It was deemed desirable to represent conditions from a single specimen in order to avoid any possible confusion arising through individual variations, since recent studies on such forms as *Phrynotettix*, *Trimerotropis*, *Hesperotettix*, etc., have shown that individuals within a species may vary as to certain features of their chromosomal constitution, but the conditions in each individual remain constant. In the second place, a smear was used in order to insure the presence of whole complexes in each cell and thus to avoid the difficulties incident to the study of sections, where most of the cells are cut and distributed into two or more sections with frequent

disarrangements or cuts in the chromosomes. Some distortions naturally arise as a result of the smearing process, but the preparation used was more than usually free from such disturbances and in this case the advantage gained through the study of whole cells far outweighs the disadvantages arising from the smearing process.

In plate 1 all of the chromosomes in each cell are represented. It is believed, therefore, that, although identifications of any chromosome in any cell might sometimes prove doubtful, the chances for error, with all members present, is reduced to a minimum. This method also has its disadvantages, because only occasionally are all of the chromosomes in any particular cell in favorable positions for drawing.

*b. The post-spireme chromosomes.* The post-spireme stages have received the most attention and are represented on plate 1. As a glance at the plate will show, there are nine chromosomes in the reduced series. This number has been reported for all the species of the genus which have been studied except the doubtful member reported by McClung ('14) and which he now thinks may be *Circotettix* in which there were eleven. In plate 1, the chromosomes of eight cells are represented, those from each cell being arranged in a vertical column according to size, with the smallest at the bottom. The cells are lettered A, B, C, etc. Each horizontal row therefore presents examples of a particular chromosome, and successive stages in development from the end of the spireme stage to early anaphase can be read from left to right. These horizontal rows correspond in developmental stages to figures 62 to 65, plate 6, of the paper on *Phrynotettix*.

Of the nine chromosomes in the first spermatocyte one is the unpaired accessory chromosome (no. 3); the others are tetrads, consisting of three pairs of atelomitic (nos. 7, 8, and 9) and five pairs of telomitic chromosomes (nos. 1, 2, 4, 5, and 6). Column A represents the chromosomes of a cell at the stage when the spireme of the growth period has just become distinguishable as individual segments, each segment being the direct forerunner of a tetrad (except the accessory, no. 3). Each of the segments is

an attenuated thread with a longitudinal cleft, and more or less coiled or bent, according to its length. The longitudinal cleft is what has been called the primary longitudinal split.

In cell B, a second split is seen at some points, where four threads are visible (e.g., at *a*). In cell C all of the segments (except no. 1 and the accessory) have the four chromatids, indicating that the secondary longitudinal split has become complete. The other cells, D to H, represent the farther progress of chromatid transformation and condensation, through characteristic tetrad figures, to the metaphase or early anaphase of the first maturation mitosis. As nearly as possible, all of the tetrads in cells D to H have been given the same orientation; those in cells A, B, and C could not be definitely oriented, partly because the synaptic points could not be determined and, in the case of numbers 7, 8, and 9, partly because of lack of space on the plate.

*c. Synapsis.* The point to which especial attention should be directed is that all of the autosomes, or euchromosomes, show the same fundamental conditions, whether they be pairs of telomitic or atelomitic chromosomes. All come out of the spireme as elongated threads with one longitudinal cleft visible; all develop a second longitudinal cleft at right angles to the first, giving rise to tetrads, each with four chromatids. And since, as is generally believed, each tetrad represents a pair of spermatogonial chromosomes, it would appear that each of the spireme segments in cell A represents a pair of chromosomes which have been extended axially and united side-by-side throughout their length.

I have already shown ('16) how this side-by-side union of attenuated leptotene threads takes place in *Phrynotettix*. Evidence of the same nature for *Chorthippus* is presented in figures 1 to 4, plate 3. In figure 1 a nucleus is shown with one double thread (5)—that attached to the accessory—and another thread which is double at the end nearest the accessory (proximal end) but divides distally into two single threads. Numerous single threads are visible in the other parts of the nucleus. In figures 2 and 3 a considerable number of these partially conjugated pairs of threads are shown. In none of these drawings (figs. 1 to 4) was it possible to represent all of the threads because they

are so long and so extensively curved and coiled that they appear at first glance to be hopelessly tangled and to constitute a network. It was possible, however, by careful study, to follow some of the threads for considerable distances, but others only short distances. On this account these drawings are, in part, diagrammatic.

In these lepto-zygotene nuclei there is always a tendency toward an orientation of the threads just as was found at the corresponding stage in *Phrynotettix* ('16, figs. 29, 30, 31, plate 3). This orientation appears to be confined mostly to the region near that end of the thread to which the spindle-fiber is attached (proximal end) and therefore that side of the nucleus toward which these ends are directed is called the proximal side. From this proximal side the threads run more or less parallel toward the opposite side of the nucleus, thence turning to traverse the nuclear space in various curved or convoluted courses. The amount of curving depends, apparently, on the length of the threads, though even the shorter ones have a tendency to form a loop as is indicated by the short, deeply staining thread (5), attached to the accessory chromosome in figures 1 and 4. In general, only the proximal ends of the threads exhibit orientation and this orientation, I believe, facilitates the initiation of the pairing process and causes its inauguration to occur at the proximal ends, as shown in figures 1 to 3. In the distal part of these figures most of the threads are single. The accessory (X), usually has one end attached at the proximal pole, as shown in figures 2 and 3. In figure 4 the position of the nucleus was such that the orientation could not well be shown without interfering with other features which it was desired to represent.

The nuclei in figures 2, 3, and 4 were chosen for drawing because they show, in addition to the partially conjugated threads already mentioned, certain others to which small plasmosome-like bodies, or chromomere-vesicles, were appended. These structures, (c), as will be pointed out later, mark the synaptic points on one of the pairs of V-shaped chromosomes.

The question has naturally arisen as to the behavior of these V-shaped chromosomes in synapsis. Does the process of conju-



gation begin at the apices of the V's and proceed toward the ends, or does it begin at the free ends and proceed toward the apices? The drawings shown are not entirely conclusive on this point, though very suggestive. In figure 2 the two synaptic points are marked by deeply stained knobs to which the 'vesicles' are attached. These knobs are not situated at the proximal side of the nucleus, but appear to have been pulled away from this position which they undoubtedly held in the telophase of the last spermatogonial division (figs. 7 and 8). The knobs are at the apex of an angle made by a sharp bend in the threads at that point, and while the knobs themselves stand somewhat apart, the threads appear to be conjugated for a short distance on either side of them. When I attempted to follow these conjugated threads they soon became lost in the maze of unconjugated, single threads. The appearances, however, indicate that the only parts of these threads which had conjugated at the time were those immediately adjacent to the appendage-bearing knobs, and therefore at the apices of the V's.

In figure 3 a situation is presented somewhat similar to that in figure 2, except that here conjugation has taken place on only one side of the apical knobs, the remainder of this pair of threads apparently consisting of unpaired leptotene strands. In figure 4, conjugation of this pair has gone further, but even in this case the conjugated part is confined to the region on either side of the synaptic points. These cases, together with others which have not been drawn, tend to support the idea that conjugation of the atelomitic chromosomes begins at the apex of the V, which, as the point of spindle fiber attachment, is homologous with the proximal end of the telomitic or rod-shaped chromosomes.

As soon as conjugation is complete, all of the chromosome threads appear double, as shown in figure 5, which represents the pachytene stage. In this nucleus, the usual orientation of the chromatic elements has been lost. There is some tendency for the threads to be centered toward the side of the nucleus which is uppermost in the drawing, but the accessory chromosome (X), which is customarily attached at the proximal pole

of the nucleus, is here removed a considerable distance, and separate from the deeply stained loop (5), to which it is usually attached. This deeply-staining thread is found in the later stages to be that of chromosome 5 (see plate 1) and it very commonly lies near, and often attached to, the accessory. This association may be seen in figures 1, 4, and 6.

Figure 6 represents a nucleus at about the same stage as cell A on plate 1 and shows the separate spireme segments. Each of these has but one longitudinal split, but each represents a tetrad. The segments are numbered according to the arrangement on plate 1. A series of successively older stages may therefore be recognized, beginning with figure 1, extending through figure 6 on plate 3, and continuing through cells A to H on plate 1.

The series of stages just mentioned constitute, it seems to me, a sufficient body of evidence to indicate that in *Chorthippus* parasynapsis is the rule.

*d. Chromatid movements.* Attention may be directed to the different rates of change in the chromatid relationships experienced by different chromosomes in the same cell and by the same chromosome in different cells. The typical form of the metaphase chromosome of the telomitic type seems, in this species, to be the rod extended parallel to the spindle axis. Such a form is taken by chromosomes 1, 2, 4, and 6 of cell G on plate 1. Although number 5 has not been pulled out in the direction of the mitotic poles sufficiently to make it a rod, it is readily apparent that only a small amount of movement of the oppositely directed pairs of chromatids would produce the forms seen in chromosomes 4 and 6. As an example of the different degrees to which the chromatid movements have brought about an approach to the extended-rod condition of the metaphase, attention may be called to cell E, where chromosome 4 seems to be lagging much behind the other chromosomes, though in cells F, G, and H, such is not the case. In cell D, on the other hand, chromosomes 2 and 5 seem to be farther advanced toward the metaphase-rod condition than any of the others.

When we examine a particular chromosome as it appears in different cells we do not always see a consistent progress in development in the successively later stages. Chromosome 4 in cell E seems to be in about the same condition, so far as chromatid movement is concerned, as it is in cell C; that is, either one could, by the simple rotation of the free arms, assume the condition seen in cell G. However, it is just possible that in cell D a valid intermediate stage is represented and that this chromosome in the condition found in cell C would have to pass through a stage such as that in cell D before reaching the condition seen in F or G.

*e. The planes of the first division.* In the absence of well-marked polar granules it is impossible to determine which are the proximal, or synaptic, ends of the chromatids, but if we may assume for the time being that the ends at the left in cells B to E are the synaptic ends, we may trace for chromosome 4 successive steps as follows: In cell B the longitudinal split on the right (the only one visible for two-thirds the length of the tetrad) is probably the primary longitudinal split. The split just forming at the left and continued to the point *a* is probably the secondary split. In cell C, then, we may consider that the formation of the secondary split has been completed and that the chromatids have separated along this split almost to the distal end. Such an element might remain in this condition, condensing through a stage such as is shown in cell E and finally opening out to form an element in the metaphase like the one in cell G. In cell D we may have a stage in which the secondary split has become complete but the separation along its plane has not. If the progress of separation along this plane were to be impeded by gradual condensation, this stage might be followed by a cross formed by the proximal ends (left) of the two pairs of chromatids becoming extended in opposite directions in the same plane (as shown in chromosome 2, cell D), which cross might reach the metaphase in some such form as shown for chromosome 5 and 6, cell F, or even chromosome 4, same cell. A similar series of stages occurs for chromosome 2 in cells B, C, and D. Series of movements such as have been suggested would corre-

spond in all essentials to the series of changes found to occur for chromosome A in *Phrynotettix* (see '16, fig. 62, plate 6), and would indicate an equational division. Other interpretations, indicating a reductional division are, of course, possible, and cannot be excluded.

In chromosome 6 a similar series of stages may be traced. In cell B the split running through most of the length of the element from the right is probably the primary split, while that just beginning at the left and extending to point *a* would be the secondary split. A continuation of the secondary split and a separation along its plane would give rise to such a stage as that seen in cell C, or, if the separation were delayed, to that in cell D. A rotation of the arms of the tetrad in cell C would give rise to a stage such as is shown in cells E and G, while a similar rotation of the arms of the example in cell D would give rise to the condition seen in F.

It is regrettable that the polar granules, which do occur to some extent, are not sufficiently differentiated by the staining method used to make the orientation of the tetrads certain. In sectioned material stained with iron-haematoxylin polar granules are, in fact, better differentiated than in the safranin stained smear, but in this instance little study has been made of sections, where identification of all the chromosomes is not so certain.

In the case of the pairs of atelomitic chromosomes (nos. 7, 8, and 9) variations in rate and extent of chromatid movement are not so apparent in the examples drawn on plate 1. Such variations do exist, however, as will be seen by comparing number 8 with three 'rings' in cell C, and two 'rings' in cells D, E, and F, and figures 9 to 14, plate 3.

*f. The apical constrictions and the chromomere-vesicles.* In these atelomitic chromosomes the synaptic point is in many cases very clearly marked by a constriction, as Robertson ('16), has emphasized. This constriction (point *b* in the figures, plate 1) is not visible in all cases, as is indicated in the figures. Chromosome 9 in cell E for example, shows scarcely any evidence of a constriction, although it is plain enough in chromosomes 7



and 8. In cell F chromosomes 7 and 9 show the constriction, while it is not apparent in chromosome 8. Again in cell G chromosome 9 did not reveal the constriction as did chromosomes 7 and 8. All three show evidences of a constriction in cell C.

In connection with this synaptic point, as already mentioned, there is a small plasmosome-like appendage, or chromomere-vesicle (marked *c* in the figures), which seems, in the earlier stages at least, to be as constant in its occurrence as is the constriction just described. It disappears in the later tetrad stages as such structures usually do. Similar appendages are occasionally seen attached to the synaptic points of chromosomes 7 and 8, as is indicated for the former in cell D and for the latter in cell A, but their occurrence on these chromosomes is rather infrequent.

It will be noticed that only one appendage is to be seen on a single chromosome in the figures on plate 1. In earlier stages, however, there are two such bodies as is indicated in figures 2, 3, 4, 6, 7, 8, and 9, plate 3. Figure 5 shows the two partially fused together and many times they appear to be entirely fused at these stages. It could not be determined whether the single appendage in plate 1 and in figures 10 to 14 on plate 3 represent two fused together, or whether one of them disappears before the other. The latter possibility is suggested by a constant difference in size in all cases where two are in evidence and by the fact that the single ones in figures 10 and 11 do not appear to be equal in bulk to two such as are shown in figure 9. In figures 12, 13, and 14, they appear larger, but these figures are taken from sections from another individual, which fact may account for their larger size. That these structures are constant elements of this pair of atelomitic chromosomes is well illustrated by the spermatogonial telophases shown in figures 7 and 8. In each case the 'vesicle' is attached at the apex of a V-shaped chromosome with a marked disproportion between the lengths of the arms, comparable to that seen for chromosome 9 on plate 1. In figure 8, the smearing process has resulted in a slight distortion of one of the atelomitic chromosomes (at the left) to which an appendage is attached. It seems fairly safe to



regard these chromomere-vesicles as diagnostic for the apices in this largest pair of V-shaped chromosomes.

In the figures from sections (figs. 12 to 14, plate 3) the appendage is attached to only one member of the pair of knobs which mark the synaptic point, and, further, the attachment is always to the knob nearer the middle of the chromatid. In the smears this position is not so evident owing to the lack of definite knobs showing at the synaptic points. However, chromosome 9, cell A, does show such knobs and it will be observed that the appendage is attached to the one nearer the middle of the chromatid. Chromosome 8, cell A also shows such a relationship. The possible evidence that these structures furnish toward an understanding of the planes of first spermatocyte division will be considered in the discussion.

*g. The accessory chromosome.* The accessory chromosome does not require description or discussion in this paper, but attention may be called to the vesicular appendage attached to it. This appendage is similar to those just mentioned which are attached to the atelomitic chromosomes. This structure seems to have been overlooked by all those who have studied this form, except Davis ('08).

*h. Summary.* In summarizing the observations on *Chorthippus* we may call attention to the following:

1. The number of chromosomes in the reduced series (first spermatocyte) is nine, consisting of one unpaired accessory chromosome and eight pairs of autosomes or euchromosomes. Of the latter there are three pairs of atelomitic and five pairs of telomitic chromosomes.

2. The leptotene threads conjugate two-by-two throughout their length during the zygotene, or lepto-zygotene, stages. They remain conjugated through the pachytene stage and until the tetrads are formed. This condition of parasynapsis holds for all the euchromosomes, both telomitic and atelomitic.

3. The process of parallel conjugation (parasynapsis) appears to be inaugurated at the proximal ends of the leptotene threads as was the case in *Phrynotettix*, and it appears to begin at the apices of the atelomitic chromosomes.

4. A constriction occurs at the synaptic points in most of the tetrads of the atelomitic chromosomes.

5. A small plasmosome-like appendage, or chromomere-vesicle, is attached to the conjugants of the largest pair of atelomitic chromosomes (no. 9) at the apex of the V (synaptic point). A similar appendage is occasionally found at the homologous point on the other two atelomitic pairs, and occurs very constantly on the accessory chromosome.

*B. Trimerotropis suffusa*

*a. Introductory statement.* *Trimerotropis* belongs to the subfamily Oedipodinae of the family Acrididae. A discussion of some systematic problems connected with this genus will be found in the paper by Dr. Carothers ('17). The material from which the drawings were made consisted of a single smear made by Dr. Carothers and loaned to the writer for study. This individual corresponds to her number 34 (plate 6) ('17) and cell E on my plate 2 is the same as that drawn by her. Other individuals were studied but no drawings from them are used in the plates.

The reasons for using a smear preparation in this case are the same as those already stated in connection with *Chorthippus*. This smear was fixed in Flemming's solution and stained by Flemming's tri-color method. The chromatin was not so deeply stained in this smear as it was in the smears of *Chorthippus*, and this difference appears in the drawings.

*b. The post-spireme chromosomes.* As Carothers has found, the reduced number of chromosomes in this genus is twelve. Occasionally, however, supernumerary chromosomes occur, as she discovered, and one such supernumerary occurs in this individual, making the number of first spermatocyte elements thirteen instead of twelve.

The arrangement of the chromosomes in plate 2 is the same as for *Chorthippus* in plate 1. Each vertical column represents the complete series of chromosomes from a single cell arranged in the order of size with the smallest at the bottom.

The differentiation on the basis of size is not always easy to make. This difficulty is made more apparent by the differences between the order used on this plate, where the accessory is made number 3, and the order used by Carothers, who made the accessory number 4. Such differences, however, are without significance so long as other means of identification are available to obviate confusion. The supernumerary is placed at the top of the plate to avoid any possibility of its becoming confused with the smaller tetrads. From left to right are shown successive stages in the transformation of the spireme segments to tetrads, and their condensation, final orientation, and partial division in the spindle of the first maturation mitosis.

A glance at the metaphase figures shows that the complex of this individual consists of four pairs of atelomitic chromosomes (nos. 7, 10, 11, 12), five pairs of telomitic ones (nos. 2, 4, 5, 6, 9), two heteromorphic pairs (nos. 1 and 8), the atelomitic accessory (no. 3), and the supernumerary. In other individuals of the species the relative numbers of pairs of atelomitic, telomitic, and heteromorphic pairs may be different, as found by Carothers and described by her.

It is not possible in every case always to identify each chromosome in a cell. This is due to similarities of size and organization. Chromosomes 11 and 12, for example, appear very similar in size and organization in the earlier stages, so that it is not always possible to tell which is which. In the metaphase and anaphase, however, these two are distinguishable by the different proportions between arm-lengths of the V's. Number 12 has the greatest disproportion between arm lengths; in number 11 the disproportion is less, and in number 10 still less. The proportions between arm-lengths for these atelomitic chromosomes can the more readily be determined by reason of the occurrence in many of the metaphase examples of constrictions or transverse clefts at the points of spindle-fiber attachment. Such clefts are to be seen, for example, in cells E, F, and G for chromosomes number 11 and 12, and cells E and F for number 10. Such clefts or constrictions (marked *b* in the figures) can sometimes be distinguished in the earlier stages

as, for example, one cleft for number 12 in cell D, two clefts for number 11 in cell D, two clefts for number 10 in cell C, and in the case of the atelomitic member of number 8 the cleft shows very plainly in cell D. Clefts are likewise indicated for number 7 in cells C and D, and still more plainly in the metaphase cells, E and G. These clefts or constrictions appear to correspond in all respects to those found in the atelomitic chromosomes of *Chorthippus*.

Chromosomes 5, 6, and 7 are somewhat similar in organization, each possessing polar granules at both ends. This gives rise to some uncertainty as to identity, especially in cells B and C. In cell A the differences in length are more apparent.

Number 4 in cells A, B, and C, and number 8 in cells A and B are readily identified by the peculiar clusters of granules (c) attached to them. In the later stages these disappear and this gives rise to some difficulty in identification in the case of chromosome 8. Number 4, however, possesses an additional means of identification by reason of its staining power which is constantly greater than in the other euchromosomes.

In spite of the difficulties of identification, it is felt that with all the chromosomes present in each cell, and with a careful consideration of the size and structure relations of the various chromosomes, very few mistakes in serial arrangement have been made.

*c. The evidence on synapsis.* The evidence for parasynapsis, as presented on plate 2 is of the same nature as that for *Chorthippus*, and consists in the facts: (1) that all the euchromosome pairs, irrespective of the point of spindle-fiber attachment, come out of the growth period as longitudinally split, attenuated threads, and (2) that from these split threads tetrads arise by the formation of the secondary longitudinal split. Figure 21, plate 3, gives the evidence for parasynapsis as it occurs in the lepto-zygotene stages and the conditions shown are quite similar to those already described for *Chorthippus* and shown in figures 1 to 4. Figure 21 does not represent nearly all of the chromatin threads in the nucleus and is partially diagrammatic. However, the condition indicated—namely, the presence of double threads

with one end at the proximal pole of the nucleus and the other end separating distally into two single threads—was clearly seen for a number of those represented and indicated for the others. The deeply staining body near the accessory (X) is regarded as the supernumerary chromosome. In this stage in this material none of the atelomitic chromosomes could be identified and, while the tendency for conjugation to begin at the proximal ends of the threads is clearly indicated, the behavior of the atelomitic chromosomes could not be followed.

*d. Chromatid movements.* Differences in rates of development, or progress toward separation at anaphase, in different cells find some striking examples in the series drawn on plate 2. Chromosome number 1 seems to be lagging somewhat behind the others in cells B and C, though in cell D it is as far advanced as any. In cell D, number 2 is not so far along toward its typical metaphase condition as it is in cell C. The same relations appear to hold for chromosome 5 in these two cells, yet all of the chromosomes in cell D are more condensed and therefore presumably nearer metaphase than they are in cell C.

In the metaphase there are also some striking differences. Number 4 in cell G is very far behind the other members of the complex and seemingly little advanced in chromatid movement beyond the condition seen in cell A. In cell F, too, separation is not so far advanced for this chromosome as it is in cell E. Cell H is in early anaphase, and cells E, F, and G are at about the same metaphase stage, yet chromosome 9 is not so far advanced toward complete separation in F as it is in E, and in E it is not so far along as in G, yet in G it seems further along than in H. Again number 6 is lagging in cells F and G, as compared to the conditions in E and H. The examples of this chromosome in cells F and G are at about the same stage, but in G a ring exists through the contact of the ends of the arms at the right which are seen to be free in cell F.

All these differences seem to indicate that the extent of chromatid movement is variable, not only for different chromosomes but also for the same chromosome in different cells, so that in some cases the movement which ends in the separation accom-



plished in the anaphase may be well advanced before the element reaches the metaphase, while in other cases these movements are largely deferred until after the element has been oriented in the metaphase spindle.

There are some pronounced differences in shape of the atelomitic chromosomes in the metaphase. In the case of chromosome 12, for example, a different shape is seen in each of the cells, E, F, G, and H. It is easy to see how a simple movement of the oppositely directed dyads in cell E toward their respective poles would give rise to the condition in cell H; but no such simple movement would account for the shapes in F and G. The explanation of these various shapes, I think, may lie in the statement made above as to the variations in chromatid movement in the earlier stages. A search among the tetrad figures of these earlier stages should reveal the forerunners of the shapes seen in metaphase. In cell C, for example, is a condition of chromosome 12 which would readily give rise to that in cell E if the shorter arms of the V's are toward the right and the apices of the V's are pulled out toward opposite poles. Similarly for chromosome 11, the tetrad in cell C might easily take its place in the spindle in the form shown in cell E and the tetrad of number 10 in cell D could, by orientation, assume the shape in cell E. In cell D, on the other hand, occurs a condition of number 12 which in many respects is similar to that in cell F. The chief difference is that in the latter the short arms of the V's have been reflexed toward the longer arms. The tendency for the shorter arms to bend back in this way when they are free is not infrequent among the atelomitic chromosomes, as shown by McClung ('14). A somewhat similar metaphase of number 11 or 12 from another cell is shown in figure 18, plate 3. Figure 17 shows an earlier tetrad which could give rise to it by the separation of the ends on the right and their flexure backwards toward the left.

None of the earlier stages for chromosome 11 or 12 as seen on plate 2 indicate a condition which could by any simple movement give rise to the shapes seen in cell G. Here the curious condition is found of the shorter arms remaining in contact,

while the longer arms have become free. From the standpoint of simple mechanics it would be difficult to understand how a tetrad such as is seen in cell C, for example, could give rise to the condition in cell G. It would be natural to suppose that the crossed longer arms (at the left) would require a greater amount of force for separation than the shorter arms in contact at the right; the pull of the spindle fibers would be expected to be exerted more strongly on the shorter ends. The conditions in cell G are the exception rather than the rule, and I have not found an earlier tetrad which showed the longer arms free as they are in this cell. However, in figure 19, plate 3, there is a ring-shaped tetrad which, by the mere separation of the lightly attached ends of the long arms at the left, would provide a condition analogous to that in figure 20, which resembles those in cell G, plate 2. A condition which is the reverse of that in figure 20 is shown in figure 16, where the short arms are free. Such a shape could readily arise from that in figure 15 by a little more separation along the plane between the arms at the right and their flexure at the point of spindle-fiber attachment.

It might be remarked that one cannot be certain whether chromosome 11 or 12 is represented in figures 15 and 17 because the synaptic points are not indicated by clefts as they are in figure 19. Figure 16 is doubtless number 12, with its great disproportion between arm lengths, while figure 20 is more probably number 11. Figure 18 is problematical.

In cells E and G chromosome 7 (plate 2) seems to have the shorter arms united and the longer arms free, while in F the longer arms are attached to each other and in H they have been the last to separate. In cell E the difference between arm lengths seems much less than in the other cells. This is to be accounted for by the foreshortening of the longer (free) arms and by the stretched conditions of the attached shorter arms, shown by their smaller diameter.

As a net result of such observations one may conclude that the movements of the constituent chromatids of a tetrad are subject to great variation and that with respect to the separation of the ends, there is sufficient evidence to show that either the

longer or the shorter ends may become free in advance of the other end. Just why the chromatids should assume the varying relationships to each other in similar stages of the tetrad transformation period is of course not easily determinable, but the metaphase figures seem to be derived from similar ones in the earlier stages without much movement of the chromatids during the later condensed prophases, nor until the forces in metaphase and anaphase are at work. One may suppose that the movement of the chromatids takes place at different rates in different cells and that, as condensation in the later prophases progresses, such movements are retarded; the result being that whatever stage in transformation has been reached in a late prophase is the one in which the element enters the spindle. The further movements then must be the result of the forces in operation during metaphase and anaphase.

*e. The planes of the first division.* In Chorthippus, as described on pages 480 and 481 the evidence seemed to favor an equational division for chromosomes 2, 4, and 6 (plate 1) which are telomitic. Evidence of equational division among the telomitic chromosomes of Trimerotropis can be found on plate 2. Chromosome 2, for example, as it appears in cell A, has a very characteristic arrangement for the parts of this element. It has very pronounced polar granules at both ends, those at the distal end being the larger. That the larger granules represent the distal end is shown very well in the metaphase figures E, F, G, and H. A further indication that these larger granules are at the distal end is their behavior toward each other. In all of the cells studied which were at stages corresponding to cells A and B, these large granules stood widely apart from each other. The example in cell A shows this especially well. It would hardly be supposed that the proximal ends of a pair, being the first to conjugate, would behave in this manner, while the distal ends might easily fail of complete pairing. Now, since it is possible to determine which end is proximal and which distal, it is also possible, I believe, to trace the entire series of changes into the metaphase. In cell A the primary split is visible at the distal end (at the right in the figure) of this chromosome, but

not through the middle. A split is seen in the proximal end (at the left) but it cannot be determined whether this represents primary or secondary split; in cell B, however, both splits are easily seen and it is further observable that the separation at the proximal end (left) is along the plane of the secondary split. The tendency for the proximal granules to diverge with further separation along the secondary split is seen in cell C. In cell D the relation of the chromatids is much the same as in cell B but they are more condensed. In cells E, F, and H, as I have already noted, smaller knobs or enlargements occur at the proximal ends, while a bulging at the middle of the oriented tetrad indicates the larger distal granules. It seems fairly safe, then, to assume that a tetrad in the condition seen in cell C would become oriented in the metaphase without further change in the relationships of the chromatids, except their further separation along the plane of the secondary split. There seems to be good reason for concluding, therefore, that division is equational for this chromosome.

There is also some evidence that chromosome number 4 divides equationally in this first division. In the metaphase cell E the horizontal components of the chromatids on the right side of the tetrad have stained differently from those on the left. They failed to retain the stain so well and appeared finely granular, as indicated in the drawing, in contrast to the compact, deeply staining chromatids on the left. It is reasonable to suppose that those two chromatids which show this peculiarity belong to the same parent chromosome, and, therefore, that halves of chromosomes are being separated in this division, constituting an equational division. Attention may be directed to the cluster of granules attached to this chromosome. In the examples in cells A, B, and C, plate 2, it cannot be determined just what relation this appendage bears to the chromatids. In figures 22 and 23, plate 3, however, it is plainly indicated that the structure is attached to a chromomere of only one of the conjugated threads. Presumably this condition holds true for all stages. If that be true, then these appendages may be at the distal end, if it be permitted to associate the peculiar stain-



ing condition of the example in cell E with the presence of this appendage in the earlier stages.

In the cases of chromosomes 5, 6, and 9, there is no method of distinguishing distal from proximal end in the earlier stages and therefore no deductions can be drawn as to which plane of separation prevails in metaphase.

For the heteromorphic pairs of chromosomes (nos. 1 and 8) there seems to be little doubt that the first division is a segregating one, because of the striking difference in shape of the segregating parts. In this respect they are analogous to the unequal pairs found in *Phrynotettix* and other forms which divide reductionally in the first division.

*f. Additional observations on chromosome organization.* Some additional points of interest on the subject of chromosome organization may be indicated. In the first place outstanding chromomeres are much more abundant than in *Chorthippus*, and this is particularly true of those occurring at the ends of the chromosomes. These polar granules (chromomeres) have already been noted in chromosomes 5, 6, and 7, but they are also prominent in the earlier stages, at least, in numbers 2, 4, 8, and 9. The terminal chromomeres are less prominent but easily distinguishable in number 10, still less so in numbers 11 and 12. In most cases, with the gradual condensation of the entire chromosome, these prominent chromomeres become difficult to detect in the later stages (cell D, *e. g.*). In the case of number 2, however, the polar granules are relatively very large and they continue to be recognizable even in the metaphase (cell E).

Secondly, the presence of the appended bunches of granules (c) on chromosomes 4 and 8, already noted, may be considered further. As characters for identification of these chromosomes they have been mentioned on a previous page. An examination of the pachytene stages shows these appendant structures to be prominent. Figures 22 and 23 (plate 3) show them (c) attached to their respective spireme segments. Chromosome 4 is further identified in these figures by its greater capacity for holding stain. In both of these figures the appendages appear to be attached to only one of the conjugants of each pair of threads,



and furthermore, they appear to be joined to single chromomeres in three of the four illustrations. In both cells, that end of the chromosome, near which the appendages are attached, is united with the end of another chromosome, the opposite ends being free in each case. In figure 23 the burdened ends of these two chromosomes are joined together, the appendages are in contact and, so far as could be determined, may possibly be fused at the point of contact. It will be noted that the chromomere to which the appendages are attached appears to be in about the same position with reference to the end of the segment in both cells.

A study of still earlier stages to determine whether these appendages may be double, that is, one for each conjugant, was not made. In the stages shown on plate 2 the bunches of granules were so disposed as to make it impossible to determine whether or not their attachments were restricted to one pair of chromatids. Structures which were interpreted as representing these appendages were seen in the nucleus from which figure 21 was made but were not drawn because to have done so would have confused the other features which were to be emphasized.

A third point on the subject of organization is the occurrence of the transverse clefts or constrictions at the points of spindle-fiber attachment in the atelomitic chromosomes. These clefts have been described in another connection so that it will be unnecessary to repeat a description of them.

In passing, it may be noted that the distribution of the accessory chromosome with reference to the members of the heteromorphic pairs follows the law of chance, as discovered by Carothers ('17). (See chromosomes 1, 3, and 8, plate 2.)

*g. Summary.* In this individual of *Trimerotropis* the following conditions have been found:

1. The reduced number in the first spermatocyte is twelve (plus one supernumerary). The complex consists of five pairs of telomitic chromosomes, four pairs of atelomitic chromosomes, two heteromorphic pairs, and the atelomitic accessory besides the supernumerary. These facts were all previously

determined by Carothers, and the present observations corroborate those made by her.

2. Synapsis consists in a side-by-side union of leptotene threads, which parallel association persists until the breaking up of the spireme into its constituent segments and the production of tetrads by the formation of the secondary longitudinal split.

3. The process of parallel conjugation appears to begin at the proximal ends of the leptotene threads, as was found in *Chorthippus*.

4. The great variety of metaphase shapes for the same tetrad appears to be correlated with variations in the extent and character of the movements of the chromatids with reference to each other in the earlier tetrad stages.

5. At the point of spindle fiber attachment (synaptic point) in the pairs of atelomitic chromosomes there occurs a transverse constriction, or cleft, similar to those found for the atelomitic chromosomes of *Chorthippus*.

6. Chromosomes 4 and 8 (plate 2) each bears near one end a cluster of granules which is thought to be homologous with the chromomere-vesicles seen in *Chorthippus* and other grasshoppers.

### III. DISCUSSION

On the basis of the foregoing observations it seems worth while to discuss briefly the following topics: (A) Synapsis, (B) pre-reduction vs. post-reduction, (C) chromosome organization.

#### *A. Synapsis*

In considering the subject of synapsis it may be well first to summarize the evidence gained from the study of *Chorthippus* and *Trimerotropis*, together with that previously recorded for *Phrynotettix*, and then to compare these evidences with the results recorded by others who have studied *Chorthippus* (*Stenobothrus*). An extended discussion of the literature on synapsis would be superfluous in view of the consideration given the subject by McClung ('14), Robertson ('16) and the writer ('16).

The evidence for parasynapsis as it has been presented for Chorthippus and Trimerotropis on the previous pages is very much of the same nature as that presented in the paper on Phrynotettix ('16). The chief differences are as follows: In Phrynotettix three particular first spermatocyte chromosomes were selected for intensive study because they possessed characters which made their identification possible at most of the stages concerned. For these three chromosomes stages in the transformation of the double-thread spireme segment to the tetrad in metaphase or anaphase were traced. In the present study similar stages have been traced for all of the chromosomes in both species. In Phrynotettix the leptotene and zygotene stages were carefully studied and one of the selected pairs of chromosomes traced through these stages. In Chorthippus and Trimerotropis, lepto-zygotene stages have been found which demonstrate the phenomenon of parallel conjugation, beginning at the proximal ends of the threads, just as was found for Phrynotettix.

In the latter species all of the chromosomes are of the rod-shaped or telomitic type, while in Chorthippus there are both telomitic and atelomitic types and in Trimerotropis there are, in addition to the two types named, heteromorphic pairs with one member telomitic and the other atelomitic. I think the evidence presented shows very conclusively that parasynapsis occurs for all the chromosomes of whatever type in these three species.

When we consider the papers of others who have studied the male germ cells of various species of Chorthippus (Stenobothrus) we find that not all have expressed an opinion on the subject of synapsis, and among those who have considered the subject, there are some differences of opinion.

The work of Carnoy ('85), who studied the male germ cells of Stenobothrus along with those of a large number of other Arthropods, was of a pioneer kind and did not concern itself with the subject of synapsis.

Davis ('08) was the first among those who have studied Stenobothrus to consider seriously the subject of synapsis, and

on account of a misinterpretation of the looped condition of the pachytene threads he decided that synapsis consisted in an end-to-end union. He regarded the longitudinal cleft of these threads as having arisen by a splitting of the individual chromomeres of the single leptotene threads, instead of arising from a pairing of chromomeres, as I have shown. He failed to detect the formation of the secondary split, confusing it with the primary, and also failed to notice the zygotene stages with their partially conjugated pairs of threads.

Gerard ('09), on the other hand, did notice the zygotene stage with the partially paired threads and correctly interpreted them as evidence of parallel conjugation. He regarded one of the conjugating threads as more prominent "principale" than the other "secondaire" but I have failed to find evidence for such a distinction. Gerard also noticed that pairing began at the proximal pole and progressed distally, as I have described, and further that orientation was not complete nor entirely persistent in the pachytene stages.

Meek ('11), in his first paper on the spermatogenesis of *Stenobothrus viridulus*, does not commit himself on the subject of synapsis further than to point out the reduction in number of chromosomes in the first spermatocyte and then to say (p. 12): "This reduction must be effected before the breaking up of the spireme, for I have found no evidence of lateral association of filaments after this has occurred," and then (p. 15): "Probably . . . a numerical reduction takes place as a result of lateral association of chromatin granules or masses on the reticulum of threads prior to the spermatocyte prophase of mitosis." In a later paper ('12) he concludes that conjugation in pairs actually does take place, though he offers no proof for the statement and it is apparent that he has not observed the critical stages.

McClung ('14) in considering a *Stenobothrus*-like form along with numerous other species of *Acrididae* admits the possibility of parasynapsis, but did not make a study of the earlier stages where conclusive evidence is to be found.

Robertson ('16) considers the subject of synapsis in *Chorthippus* at some length. His figures 163 to 180 represent about the same stages as those shown on plate 1 of the present paper. The evidence in the two sets of drawings is of the same general nature and consists in the facts that each of the early post-spireme segments is a slender filament with one longitudinal split and that a second longitudinal split appears in a plane at right angles to that of the first, giving rise to the tetrads. So far as this evidence goes, therefore, my work, though done independently serves only to corroborate his.

In regard to the actual process of conjugation Robertson has made no observations, but assumes, in accordance with the work of the Schreiners ('06) on *Tomopteris* and *Salamandra* and the work of Janssens ('05) on *Batrachoseps*, that, in the V-shaped chromosomes at least, the process of pairing begins at the distal ends and proceeds toward the apices of the V's. He is further led to this conclusion by finding interlocking pairs, the peculiar relations of which could readily be explained on such an assumption. He then carries the analogy over to the rod-shaped chromosomes and considers that they probably also begin pairing at the distal end.

My observations, as well as those of Gerard, show that, in a general way, the reverse process takes place, namely, that conjugation is initiated at the proximal end and proceeds distally. In the case of the atelomitic chromosomes the evidence is not quite so conclusive, but that shown in figures 2, 3, and 4, plate 3, indicate that here conjugation begins at the apices of the V's, and proceeds distally. However, I am inclined to believe that the progress of pairing may not always be uniform along the whole length of the threads, but that chance association of the distal ends of a pair would result in their union, even if the region between the ends and the apices had not completely united. Such a condition is shown very clearly in the case of chromosome A in *Phrynotettix* (see '16, figs. 73, plate 6, and figs. 75 to 78, plate 7), and the interlocking of chromosomes such as that shown by Robertson in his figures 163 and 177 could be explained by such behavior.



In conclusion it may be said that parasynapsis has been amply demonstrated for *Chorthippus* through the work of Gerard, Robertson, and myself. The evidence is just as conclusive for *Phrynotettix* and *Trimerotropis* as found by me, while Robertson found clear evidence in *Syrbula*. Besides these examples from among the *Acrididae*, parasynapsis was also found among *Orthoptera* by Otte ('07) for *Locusta*, by Morse ('09) for blattids, by Vejdovsky ('11-12) for locustids, by Stevens ('12) for *Ceuthophilus*, and by Robertson ('15) for *Tettigidae*.

*B. Pre-reduction vs. post-reduction*

The old controversy over pre-reduction and post-reduction which has agitated the minds of cytologists for so long a time seems still far from a settlement. Even without considering the subject of synapsis there has been little tendency to agreement and with the assurance that parasynapsis is the rule in a large number of animals (Wenrich, '16) the uncertainty becomes more acute. There is one thing, however, about which I believe we can be certain, and that is that there is no general rule which is followed by all chromosomes at all times, and a failure to appreciate this fact has had much to do with the present unsettled state of this subject. I have shown for *Phrynotettix* ('16) how chromosome-pair C (type  $C_1$ ) divided by pre-reduction half the time and by post-reduction half the time. In the case of chromosomes A and B the rule was post-reduction, and I believed that post-reduction was the rule for the remainder of the euchromosomes. In that paper (quoted on page 472) I was also bold enough to declare my belief that pre-reduction was the rule with the atelomitic chromosomes of *Chorthippus*. My greater familiarity with the atelomitic chromosomes since my study of *Chorthippus* and *Trimerotropis* has somewhat shaken my faith in some of the criteria used to determine this point. I am strongly inclined to believe that the mode of division in the first spermatocyte cannot be determined with absolute certainty in the absence of recognizable differences between the two conjugants of a pair.

In this connection it may be of interest to review the evidence on this subject as found in the three forms that I have studied (*Phrynotettix*, *Chorthippus*, and *Trimerotropis*), and then to compare the opinions of some other investigators, especially those who have studied *Chorthippus* (*Stenobothrus*). It will be necessary to consider only the chromosomes of the first spermatocyte, for the behavior in this first division will govern the subsequent behavior in the second spermatocytes.

In the case of the telomitic chromosomes of *Phrynotettix*, unequal chromosome-pair C, type C, divided half the time reductionally and half the time equationally. Chromosome-pair B, unequal type, always divided equationally while the equal type behaved in precisely the same manner and presumably also divided equationally. Chromosome-pair A divided equationally with the possible exception of the ring-shaped forms, which were not traced to the metaphase and their behavior not determined.

In the case of the telomitic chromosomes of *Chorthippus* no very conclusive evidence is available, but a probable behavior leading to an equational division has been described on a previous page (480). Among the telomitic chromosomes of *Trimerotropis*, number 2 (plate 2) exhibited convincing evidence of an equational division, and good evidence for a similar type of division was found for number 4.

When we come to consider the pairs of atelomitic chromosomes, a decision as to pre- or post-reduction is not so easily reached. The evidence in relation to the chromomere-vesicles found in *Chorthippus* may be made to favor pre-reduction. In plate 1, it will be remembered, whenever a vesicle occurred it was always single and attached to but one pair of chromatids. This single one might be accounted for in one of two ways: either the two that are found in the earlier stages (figs. 2, 3, and 4, plate 3) have fused together to form a single body, or there may be a normal difference between the two causing one to disappear before the other. In all cases where two are seen, one is slightly larger than the other. This is well shown, for example, in figure 9, plate 3, which presents one of the few cases encountered in which both vesicles appeared at so late a stage. Figures 7 and 8

also show this difference. In figures 10 and 11, the vesicle that is present does not appear to be any larger than the largest one in figure 9, and this fact lends support to the idea that the smaller one disappears early instead of fusing with the larger. The cases shown in figures 12, 13, and 14 are from section of another individual and can therefore not be discussed in this connection. If we assume, then, that the pair of chromatids to which vesicles are attached in chromosome 9, cells E and F, and in chromosome 7, cell D (plate 1), for example, represent one of the conjugants, then the evidence seems clear that a reductional division should take place.

On the other hand, if it be assumed that the two vesicles have fused to form one, then one of them must have broken loose from its former attachments when the pairs of chromatids separated, as they do in the tetrads, whether or not the separation is along the primary or the secondary plane. When it is considered that these structures are eventually destined to disappear in the later tetrad stages, as shown by the fact that many of the tetrads observed in the stages represented by cells C to E (plate 1) do not show any vesicles, the assumption made above does not seem unreasonable. But, if these vesicles are to be regarded as shifting in their attachments, then it might be objected that such structures offer uncertain bases for deduction. On this account, deductions made on the basis of the behavior of these vesicles in the later tetrad stages may have to be considered as not entirely satisfactory. Nevertheless, because of the constancy of occurrence of these bodies throughout the pachytene stages, and the constant difference in size between them, as already mentioned, the most reasonable conclusion would appear to be that the one remaining vesicle, as found in plate 1, and figures 10 and 11 on plate 3, is attached to one conjugant and that therefore, a reductional division is foreshadowed.

We may attack this problem of reduction in the atelomitic chromosomes from a different angle. In chromosomes 2, 4, and 6, plate 1, a probable behavior of telomitic chromosomes was described which paralleled that of chromosome A in *Phrynotettix*,

and consisted in a first separation (though sometimes very slight) along the primary split, followed by a progressive separation of a more pronounced nature along the plane of the secondary split. When we attempt to find an analogous behavior in the atelomitic chromosomes we meet with difficulties. If, however, we follow the example of Robertson ('16) and consider each of the atelomitic chromosomes as composed of two telomitic ones joined at their proximal ends and then consider that the behavior of these component rods is analogous to that described above for the telomitic chromosomes, we would have to conclude that division of these pairs of V's was equational.

If we carry this idea of the compound nature of the atelomitic chromosomes over to *Trimerotropis*, further difficulties await us. Robertson uses the idea of compound chromosomes in *Chorthippus* to indicate that the real number of chromosomes is 23 ( $\sigma$ ) instead of the apparent 17 ( $\sigma$ ), and that, consequently, *Chorthippus* possesses the number typical of the *Acrididae*. If we assume that the atelomitic chromosomes of *Trimerotropis* also are compound then we should have 34 as the total number of telomitic chromosomes in this particular individual (see p. 506). However, if we are to consider the number of chromosomes in the *Acrididae* to be constant or nearly so we must conclude that the atelomitic chromosomes of *Trimerotropis* are not compound and therefore we cannot carry over the analogy of behavior from the possibly compound chromosomes of *Chorthippus*.

On the other hand the behavior of the chromatids may be considered as variable, so that at one time separation in any pair of atelomitic chromosomes could be along the primary plane and at another time along the secondary plane. In this connection, consider for a moment figure 11, plate 3. Here, there are three successive 'rings' with a potential fourth at the right—that is, if the two free ends were in contact, as they are at the left. Each succeeding ring is in a plane at right angles to those adjacent. If we may number these rings 1 to 3 beginning at the left and consider the incomplete ring on the right as number 4 it will facilitate discussion. We might consider either plane as



primary or secondary but let us assume that number 3 has the chromatids separated along the primary plane, with the vesicle attached to the chromatids of one conjugant. This tetrad is at a stage early enough so that it could be expected to undergo chromatid movements before it became condensed and oriented in the metapase. If ring 3 has arisen from a separation along the primary split, then the free ends (no. 4) must have separated along the secondary split. So, also, number 2; while number 1 would have separated along the primary split. Now the question may be asked if there is any reason why the further separation of chromatids should prevail along one plane rather than along the other. Does not, for instance, the greater extent of the separation in ring 2 and the smaller size of ring 3 indicate that further movement is likely to be in the plane of separation of rings 2 and 4 which we have assumed to be the secondary plane? Would not such a process give us a condition similar to that in figure 10 with the long free arms at the right having opened out beyond the apical point? Again, could not separation proceed to the enlargement of ring 3 at the expense of 2 and 4 and give rise to such a form as occurs in figure 14?

Similar situations are presented in the series from *Trimerotropis* in figures 15 to 19. In figure 15, so far as can be observed, there would be just as much chance of separation along one plane as the other, since we cannot recognize the synaptic point in this element. In figure 17, the middle ring might become enlarged so as to produce a condition such as is shown in figure 19, or the ends at the right might be separated and reflexed toward the left as indicated in figure 18, as previously mentioned (p. 488). In any case the primary cannot be differentiated from the secondary plane of separation, and in many ways it seems as reasonable to assume one as the other as likely to prevail in anaphase.

From the heteromorphic pairs another line of argument may be developed. If we assume as established that the heteromorphic pairs in *Trimerotropis* always divide reductionally as they appear to do, we have a basis upon which to argue that the other atelomitic chromosomes should behave in the same man-



ner and thus divide reductionally. As a matter of fact, chromosome 12 in cell D, plate 2, shows a constriction in one pair of chromatids (b) and not at the homologous point in the other pair. This might be considered as evidence that the constricted pair represented one conjugant which showed this peculiarity without its being shown by its partner, and that therefore a reductional division was foreshadowed. The results of Carothers ('17) also go far to support pre-reduction for the pairs of atelomitic chromosomes. She finds that for each of the chromosomes 1, and 4 to 9 inclusive one of three conditions may exist in different individuals: (1) there may be a pair of atelomitic chromosomes, or (2) a heteromorphic pair, or (3) a pair of telomitic chromosomes. Since the members of the heteromorphic pairs separate in the first division, it is reasonable to suppose that in another individual the two members of a pair of atelomitic chromosomes, both of which were homologous to the one atelomitic member of the heteromorphic pair, would also separate in the same way. And further, it might be presumed that even the telomitic homologues, when both are present in the same tetrad, would have a similar behavior. The strongest evidence, then, in both *Chorthippus* and *Trimerotropis* seems to support the pre-reduction hypothesis for the atelomitic chromosomes.

I have gone into all these suppositions and analogies for the purpose of showing how difficult it is to arrive at any general conclusion with regard to the behavior of the chromatids and the consequent plane of separation at the first division. It is my belief that only in those cases where the two conjugants of a pair can be differentially recognized can the plane of separation in the first spermatocyte division be absolutely determined for these atelomitic chromosomes.

The method of analogy is perhaps too generally used in the discussions of the subject of reduction. Gregoire ('10) attempted, unsuccessfully, to align most of the results published up to the time of the completion of his paper into an agreement on the side of pre-reduction. Davis ('08), taking the V-shaped chromosomes of *Stenobothrus* as a type, concluded that pre-

reduction occurred in them, and then by analogy, probably occurred in all the others. Gerard ('09), though somewhat uncertain, likewise concludes that the plane of separation of the atelomitic rings is along the primary plane and is therefore reductional and then says (p. 579): "Personne ne contestera cependant que tous les chromosomes doivent se comporter à ce point de vue de la même façon et que l'on est autorisé à appliquer, à tous, ce que l'on a vu d'une façon irréfutable chez quelques uns d'entre eux." I think there is danger in drawing such analogies.

Meek ('11) says he is not able to discover whether reduction occurs at the first maturation division or the next but thinks possibly both divisions are equational and a numerical reduction takes place only as a result of lateral association of chromatin granules or masses on the reticulum of the threads prior to the primary spermatocyte prophase. Meek thus failed to see the parallel conjugation (though he assumes it may occur) and does not recognize the primary longitudinal split (along which one division takes place) as the space between conjugants.

McClung ('14) mentions the difficulty in determining the plane of division in the first spermatocyte and says, p. 665-666: "It is probably true that until the relations of the chromosomes during the synaptic phase are definitely determined it will not be possible to assert unequivocally that the longitudinal axes of the paired chromosomes of the first spermatocyte represents the coincident axes of the spermatogonial chromosomes constituting it, for if there be a parasynapsis at any period there is a possibility that the doubly split thread may open out along the plane of the equational cleavage instead of along the space between chromosomes. In either event the form of the resulting chromosome would be the same." However he believes that (p. 667): "The sister halves of a chromosome remain closely united here as in other generations of cells, and that separation between parts of a tetrad is much more likely to occur along spaces between whole chromosomes." If one were to apply the reasoning in the last sentence quoted to the cases under discussion one would get variable results. Besides, I think I have sufficiently demonstrated parasynapsis and therefore the existence of the basis for the uncertainty mentioned.

Robertson ('16) is persuaded that all the autosome pairs divide reductionally in the first division. He bases this conclusion on the interlocking tetrads which he found in *Chorthippus*, the behavior of the 'bi-tetrads' in *Jamaicana* (Woolsey, '15), the segregation of unequal rods in the first division described by Hartman ('13), Carothers ('13), and Robertson ('15), and the pre-reductional division of the multiples in *Hesperotettix* and *Mermiria* (McClung, '05).

He says (p. 246): "From the instances which I have here given it seems to me the inference may possibly be drawn that all autosomal tetrads will be found to divide reductionally in the first maturation division."

In *Phrynotettix* I found one unequal pair which apparently always divided equationally in the first division and another which did so half the time. I was also able to show that Chromosome A in *Phrynotettix*, though the conjugants were of equal length, nevertheless divided equationally in the first division, and I believe that a similar behavior is clear for chromosome 2 in *Trimerotropis*. With these different facts established, the variability of behavior in the telomitic chromosomes with regard to reduction must be recognized, and, further, in the case of the atelomitic chromosomes it seems unwise to attempt to establish a general rule.

### *C. Chromosome organization*

*a. Atelomitic, or V-shaped chromosomes.* The idea of multiple or compound chromosomes was first presented for Orthopteran material by McClung ('05). In a paper in this journal Dr. McClung considers this subject in a comprehensive manner so that it will be unnecessary to discuss here more than a comparison between the chromosomes of *Chorthippus* and *Trimerotropis*.

The occurrence of a constriction or transverse cleft at the apices of the atelomitic chromosomes has been noted several times and I have already mentioned the view that Robertson ('16) has taken in regard to the possibly compound nature of the atelomitic chromosomes of *Chorthippus*. In drawing his conclusion that these chromosomes are compound, Robertson makes

two assumptions, namely, (1) that the number of chromosomes for the Acrididae is constant (i.e., 23, diploid series) and (2) that the 'achromatic bridge' or constriction at the apex represents the point of union between two telomitic chromosomes joined at their proximal ends. Were we to generalize on these assumptions and apply them to *Trimerotropis* a difficulty would immediately arise. In the first place, this form possesses the number of diploid chromosomes typical for the Acrididae (23) in spite of the presence among them of V-shaped members. If we should assume that each of these V's represents two formerly separate rods, then the total number of rods in the individual studied would be 34, as follows: for the 4 pairs of atelomitic chromosomes, 16; for the 2 heteromorphic pairs, 6; for the 5 pairs of telomitic chromosomes, 10; and for the atelomitic accessory, 2. This number (34) would be a radical variation from the supposedly constant number of 23, and in other individuals the number might be more (or less). As shown by Carothers ('17), the range in number of rods on this basis could extend from 30 (individual no. 62) to 40 (individual no. 6). From the standpoint of constancy in number, therefore, the V's in *Trimerotropis* should not be considered compound.

On the other hand, if it is to be assumed that the 'constriction' is a criterion for the recognition of compound chromosomes, then the majority, at least, of the atelomitic chromosomes in the individual studied would have to be considered compound (plate 2). It is true that the assumption of a compound nature for the atelomitic chromosomes of *Chorthippus* enables one to recognize the number typical for the group (23), but a similar assumption for *Trimerotropis* only causes a wide divergence from the typical number which is already present. Perhaps the way out of the difficulty may lie in the suggestion made by Robertson ('16, p. 221) that there are two types of V-shaped chromosomes, one type representing two rods joined at their proximal ends and the other type a bent rod with spindle-fiber attachment in a non-terminal position.

On this supposition the typical Acrididian number may be conserved by assuming that the atelomitic chromosomes of



Chorthippus are of the compound type and that those of *Trimerotropis* belong to the other type. Considering the similarity of organization of these chromosomes in the two species, i.e., the presence of the apical constriction in the two types, it will be seen that such a constriction cannot be used as a criterion for the recognition of the compound type.

Robertson seeks to extend this compounding idea to the V-shaped chromosomes of other groups of animals. It seems to be unsafe to generalize so extensively, considering that such a generalization does not seem to hold in all cases, even among the *Acrididae*. It would appear, rather, that each group would have to be considered by itself and conclusions drawn only from a study of numerous species within a group. Such a study has been made by Metz ('14) in which case the compound nature of the V-shaped chromosomes of *Drosophila* seems to be established. But in this case, as Metz has pointed out, it is as admissible to consider two rods to have arisen from the transverse cleavage of a primitively V-shaped chromosome as to assume that a V-shaped type arose by an end-to-end union of two of the primitively rod-shaped type. As Robertson has noted, Agar ('12) found very decidedly constricted chromosomes in *Lepidosiren* and that (p. 295): "The constancy of the position at which transverse segmentation takes place indicates a constant differentiation of the chromosomes in a lengthwise direction." But he considered that: "The presence of transverse constrictions or joints in chromosomes is not, without special evidence, to be taken as an indication of bivalency or of a future division plane." This note of caution ought, I think, to be especially emphasized.

b. *The chromomere-vesicles.* The plasmosome-like chromomere-vesicles were first called 'vesicles' by Carothers ('13). In '16 I called attention to their plasmosome-like appearance, at least in certain stages, and emphasized the point, already noted by Carothers, that they are related to definite regions of chromosomes. Dr. Carothers ('17) now calls them 'chromomere vesicles' (p. 473), a term which emphasizes their relationships to separate chromomeres and distinguishes them from the plasmosome vesicles found in the spermatogonia where each vesicle



represents an entire chromosome. These structures will be considered here only as further evidences of the exact organization of each chromosome.

As pointed out in the observations, these chromomere-vesicles are attached to the chromomeres at the apices of the V's in *Chorthippus*—quite regularly in chromosome number 9, less so in numbers 7 and 8—and near one end of the telomitic chromosomes 4 and 8 in *Trimerotropis*. In *Chorthippus* one is practically always attached to the accessory.

The relationship of the vesicle to a particular part of this accessory is not readily observable in the growth period, as shown in figures 1 to 6, plate 3, but on plate 1, it is readily seen that the vesicle (c) is attached to a point near the middle but always nearer one end than the other, indicating that in this case, also, the relationship is to some definite region or chromomere of the accessory.

Whenever the chromomeres at the apices of the V's in *Chorthippus* can be distinguished in the same tetrad which shows a vesicle, it is found that the vesicle is always attached to that one of the pair of chromomeres which belongs to the longer arm of the V. This is seen especially on plate 3 in figure 9 from the smear and in figures 12 to 14 from sections. It is also seen in in chromosomes 8 and 9, cell A, plate 1.

Similarly, in *Trimerotropis*, the relationship of the granular appendage is constant in the two chromosomes to which they are attached. In plate 2 the relationships are not so definitely ascertainable, because of the more condensed condition of the chromosomes and of the granules in the cluster, but it is noticeable that the cluster is closer to the end in chromosome 8 than it is in chromosome 4. This is better seen in figures 22 and 23 in plate 3. In both cells the clusters are attached to chromosome 8 at or near the last chromomere but one, near the end and in chromosome 4 to the fourth chromomere from the end. In figure 23 the ends of chromosomes 4 and 8 are in contact. The two appendages are also in contact, but it could not be definitely determined whether or not they had fused together. Taken all in all, this brief study of these slightly-understood,

plasmosome-like chromomere-vesicles adds considerable to the evidence indicating a constant and definite organization of each chromosome.

*D. Summary of conclusions*

1. Parasynapsis occurs in all the chromosomes of *Chorthippus* and *Trimerotropis*, whether they are pairs of rods, pairs of V's, or heteromorphic pairs.

2. The pairing begins at the proximal end of the rods and at the apices of the V's and proceeds distally. It is probable that the distalward progress is not uniform, but that the distal ends may occasionally become conjugated before intermediate parts have united.

3. In the telomitic chromosomes of *Chorthippus* and in chromosomes 2 and 4 of *Trimerotropis* there is evidence that the first maturation division may be an equational one. The heteromorphic pairs probably divide reductionally. In the case of the atelomitic chromosomes the bulk of evidence favors pre-reduction, but no definite conclusions on this point seems to be possible.

4. There are probably two types of atelomitic chromosomes, one, found in *Chorthippus*, consists of compound chromosomes, representing two rods joined at their proximal ends, the other, in *Trimerotropis*, is a bent rod. This is in agreement with Robertson's ('16) suggestion.

5. Chromomere-vesicles are constant in position in the chromosomes to which they are attached, and go to support the idea that the chromosomes are constant in their internal organization.

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#### EXPLANATION OF PLATES

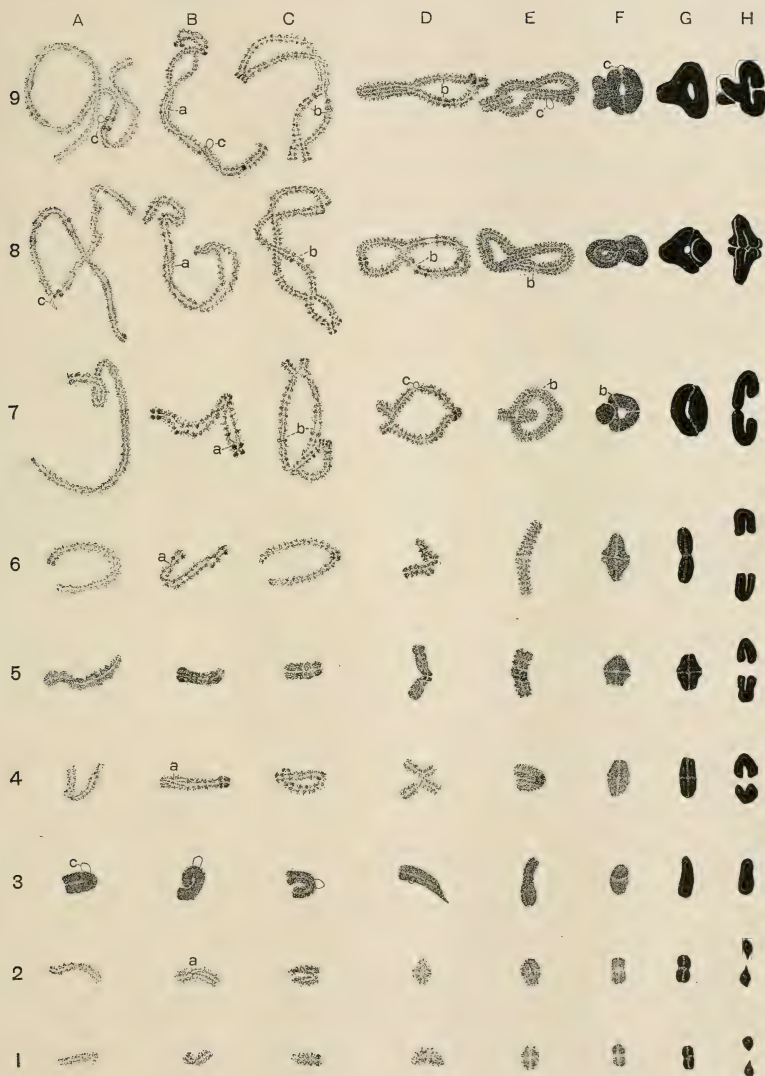
All figures in the three plates were drawn under a camera lucida at a magnification of 3000 and reduced one-half in reproduction.

#### PLATE 1

#### EXPLANATION OF FIGURES

All drawings made from a smear from a single male of *Chorthippus* (*Stenobothrus*) *curtipennis*. Vertical columns represent separate cells, each horizontal row represents one chromosome in different stages. Figures 1 to 9, at the left, refer to the separate chromosomes arranged in order of size. The letters A to H at the top refer to individual cells. *a*, Refers to four strand condition, indicating two longitudinal clefts; *b*, refers to transverse constrictions in the atelomitic chromosomes; *c*, refers to the chromomere-vesicles.





## PLATE 2

### EXPLANATION OF FIGURES

All drawings from a smear of a single male of *Trimerotropis suffusa*. Figures 1 to 12, at the left, refer to the different chromosomes arranged in order of size. Letters A to H at the top refer to separate cells. The supernumerary chromosome in the row at the top of the plate is unnumbered. *b*, Refers to the transverse clefts or constrictions in the atelomitic chromosomes; *c*, refers to appendant clusters of granules (chromomere-vesicles) in chromosomes 4 and 8.



## PLATE 3

### EXPLANATION OF FIGURES

Small numerals in figures 1, 4, 5, and 6 correspond to the numbers of the chromosomes in plate 1. The small numerals in figures 22 and 23 correspond to the numbers of the chromosomes in plate 2. *c*, indicates the chromomere vesicles; *X*, indicates the accessory chromosome.

1 to 11 From the same smear of *Chorthippus* that was used for the drawings on plate 1. Figures 12 to 14 are from sections of another individual of the same species.

1 to 4 Zygotene stages, showing partially conjugated threads. In figures 2 to 4 the apices of the atelomitic chromosome no. 9 are marked by chromomere-vesicles (*c*).

5 Pachytene stage. Chromomere-vesicles (*c*) partially fused.

7 and 8 Telophases of spermatogonia showing chromomere-vesicles (*c*) attached to chromosomes no. 9. Chromosome vesicle of accessory at *X*.

9 to 11 Tetrads of chromosome 9, illustrating variation in chromatid arrangements at about the same stage.

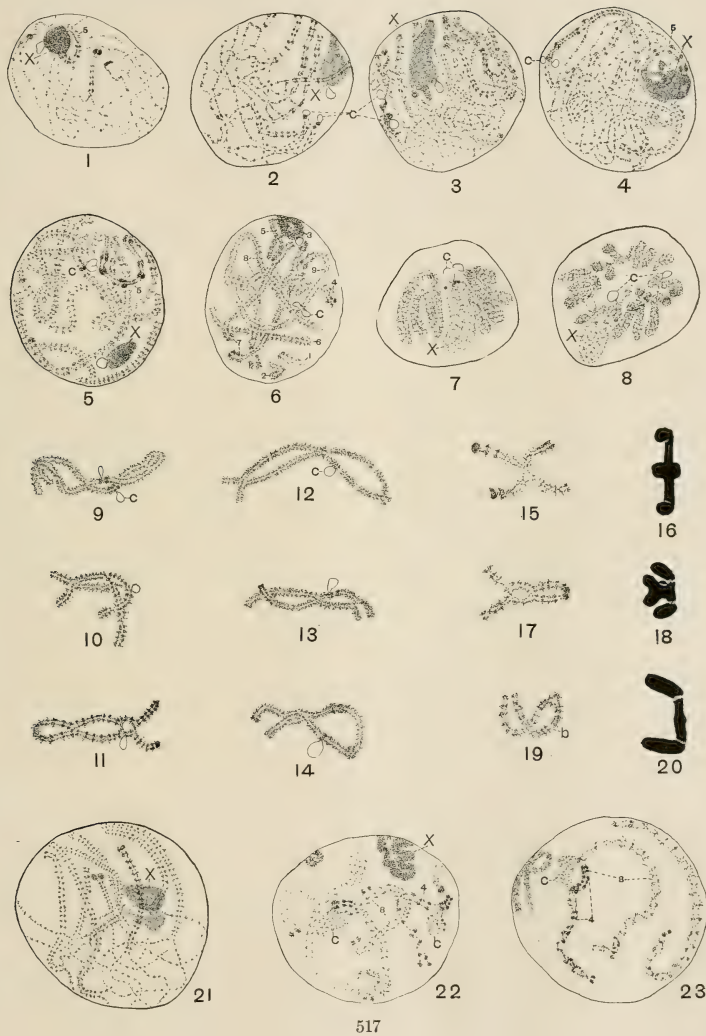
12 to 14 Tetrads of no. 9 from sections showing chromomere-vesicles and variety of chromatid arrangement.

15 to 23 Trimerotropis; from same smear as that used for drawings in plate 2.

15, 17, and 19 Prophase tetrads which may be considered forerunners of the metaphase figures 16, 18, and 20, respectively.

21. Zygotene stage showing partially conjugated threads.

22 and 23 Late pachytene stages showing chromomere-vesicles on chromosomes no. 4, and no. 8.







# THE MULTIPLE CHROMOSOMES OF HESPEROTETTIX AND MERMIRIA (ORTHOPTERA)

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EIGHT PLATES

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## I. INTRODUCTION

Under the title "The chromosome complex of Orthopteran spermatocytes" ('05) I described certain unusual conditions in these cells, among them the union between the accessory chromosome and particular euchromosomes to form multiple chromosomes. At the time my material was limited and I was able to present only a partial account. Since then I have been accumulating a large series of specimens which have been studied both

cytologically and taxonomically. In the course of this study of the more extensive series of preparations a number of facts have been determined which were obscure in the early stages of the investigation. Although it is not yet possible to present a complete study of the two genera, owing to failure to secure cytological material from all the species, there are some facts known which should be presented in order to add what has definitely been determined and to correct errors in the earlier description.

Since the appearance of my former paper ('05) upon multiple chromosomes, a number of observations upon similar structures have been reported by different investigators. Some of these, such as the ones of Voinov ('14), are not clear in their nature, but recent papers by Robertson ('16) and his student, Woolsey, ('16) have made distinct contributions to our knowledge of chromosome relations which will certainly prove valuable. These are strongly confirmatory of the view expressed in my former papers ('05, '08) regarding the persistent organization of chromosomes even in the face of apparent numerical reductions. Very striking is the discovery of a structure, the octad, which I was not then able to find, but whose existence I anticipated so strongly that I gave it a name in advance of its actual observation. The occurrence of such a multiple chromosome I am now abundantly able to confirm, as well as the steps in its formation reported by Woolsey. The underlying principles of chromosome organization, permitting the anticipation of yet unobserved conditions, stands in as striking contrast to the conception of chance association of undifferentiated masses as does the periodic law of chemists to the vagaries of the alchemists.

In this reëxamination, advantage has been taken of the unusual opportunities for a study of the taxonomic characters of the two genera, especially *Mermiria*, offered by the splendid collections at the Philadelphia Academy of Natural Science under the charge of Messrs. Rehn and Hebard. Much good is sure to come from their active interest in all that concerns the group upon which they are specializing and from their cordial and generous coöperation with other students. By their very careful studies of large numbers of excellently preserved speci-

mens they reach conclusions regarding the relationships of individuals and groups which are soundly based upon well defined external characters and upon personal knowledge of the habits and distribution of the materials. Working quite independently of them I reach conclusions regarding the relationships of individuals and groups from the study of their germ cells and, in most cases, find that there is no difference in our estimate of these relationships. Since the discrimination between nearly related forms, upon cytological characters, sometimes reveals groupings that have been overlooked by earlier taxonomists, the feeling of confidence in the validity of the theories upon which such determinations are made is much strengthened. I feel confident that a full agreement between cytology and taxonomy depends only upon the quality of the criteria of differentiation and upon the accuracy of observation on the part of the followers of these two methods of gaining a knowledge of the organization of biological units of different degrees of complexity and extent.

## II. CHROMOSOME CONDITIONS IN THE GENUS *HESPEROTETTIX*

### 1. *General observations.*

When the observations on *Hesperotettix* were first announced I had only a few specimens, and, because of unfamiliarity with taxonomic characters, my assistants failed to distinguish the species and confused the sources of the material used for study. It was only when the germ cells were examined that it became apparent that all the specimens regarded as *H. viridis* did not belong to that species. Later and more careful collections enabled me to determine that *H. viridis* and *H. pratensis* were both represented. No difficulty is now experienced in discriminating between these species by germ cell characters. Together with *H. speciosus*, these represented the full extent of my acquaintance with the genus. Although the chromosome complex was, in its major features, consistently alike in the three species, I published no further observations, because I hoped to obtain preparations from the other North American forms in order to make the presentation complete. After waiting a number of

years I am now in a position to give a much fuller account of the chromosomal characters of the genus, although I have not yet seen all the species. Much to my surprise some of the new material shows marked departures from the uniformity prevalent in all my earlier slides. As a result, some of my generalizations are now rendered invalid, but it is hoped that fuller knowledge may make possible the formulation of principles having even wider application.

At present I have preparations from five different species, but in this article I shall discuss fully only the three of which earlier mention was made, for it is only in these that multiples have so far appeared. The number of specimens in each case, except *brevipennis*, is possibly sufficiently large to be representative.

## 2. *The complex of H. speciosus*

The observations recorded for *H. speciosus* are essentially correct and need only amplification here. It will be recalled that the accessory chromosome is united with one of the tetrads in the first spermatocyte to produce a hexad element. In the spermatogonium the union with one half of the tetrad also exists and is carried over into one of the second spermatocytes. This association was invariable in all the specimens studied, affecting always the same elements. So far as could be observed the unusual relations of the accessory chromosome did not modify its peculiar character and behavior in other respects. The striking appearance of a chromosome, one part of which is condensed and safraninophilous while the other portion is granular and tinged with the violet in Flemming's tricolor, is presented to our view in prophase and telophase of the first spermatocyte. As was pointed out at the time, an apparent reduction in chromosome number occurs without there being any real difference in this respect from other Acrididae. The diploid number of twenty-two becomes the normal twenty-three when it is noted that the accessory chromosome, instead of being free, forms one limb of a V-shaped element, easily distinguished among the



twenty-one ordinary rods. That there is here a true preservation of the physical identity of each member of the complex is evident from the continued characteristic behavior of the members which are apparently fused together. The conception of genetic continuity is therefore not merely formal, but expresses the actual morphological conditions of the species. Aside from the presence of the multiple chromosome the germ cells show no marked difference from most other Acrididae.

To complete my former account of the chromosomal characters of this species the following facts may be given: In the spermatogonium appear twenty-one rod-shaped chromosomes which are generally characteristic of the Acrididae. Besides these there is a V-shaped element with arms of unequal length (fig. 9, pl. 3). That this is not a simple euchromosome is indicated by the observation that one member becomes highly vesicular during the prophases. Such a condition is uniformly characteristic of the accessory chromosome under these circumstances and is sufficient to identify this arm of the V as the accessory chromosome. During the synapsis stage there is joined to this heterogeneous pair a third member, the homologue of the euchromosome portion. In the late prophase the accessory chromosome is precocious in condensation and appears as a dense, homogeneous rod, more or less bent, joined to a granular tetrad by endwise union. The contrast is most evident in preparations by the tri-color stain. In the first spermatocyte metaphase this hexad element goes on the spindle with its long axis parallel to that of the spindle and having fibers attached at the ends of the tetrad. This brings one fiber to the point of union between the accessory chromosome and the tetrad. There are visible, at this time, eleven separate chromosomes, of which ten are quite like the ordinary Acrididae type while the eleventh is the cane-shaped multiple (complex 6, pl. 1).

The anaphase groups resulting from this division each show eleven chromosomes, but in one there is a V which has no like mate in the sister group. This is constituted of the accessory chromosome and one half of the tetrad. In the second spermatocyte into which the V goes, the nature of this is again like that

of the spermatogonium—the member which was added during synapsis having been removed in the first spermatocyte mitosis.

The second spermatocytes are accordingly of the usual dimorphic character, one with the accessory chromosome—here permanently joined to a euchromosome—and the other without it. Since the V is made up of a dyad plus the accessory chromosome dyad, it is, in effect, a tetrad, but of unusual type since its parts are not homologous. It divides here just as do tetrads in the first spermatocyte, and the anaphase shows a V going to each pole along with the ten rods. The union of the accessory chromosome with one euchromosome is therefore not lost during all the changes of the maturation period, but persists into the spermatozoon and, by it, may be handed on to the female line of the next generation from which it passes into the male line upon the following fertilization.

### 3. *The complex of H. pratensis*

In this species, as in *H. speciosus*, there is uniformly present a multiple chromosome which differs from the *speciosus* form only in the proportion of its parts. Without some more definite criterion for the homology of the euchromosomes than we now possess it is not possible to say that it is the same member of the complex with which the accessory chromosome unites in the two species. If size only were used as a basis for judgment, then it would be necessary to say that different tetrads are involved, because the euchromosomes are much larger in *speciosus* than in *pratensis*. From the fact that the accessory chromosome itself may vary considerably in different species, it would appear that size is not alone a safe indication of homology. Leaving out of consideration the question of whether it is a particular one of the eleven tetrads with which the accessory is joined, we face the concrete fact that so far as our observations have gone, there is always such a multiple element present in the germ cells of the two species.

When we come to observe the character of the multiple element in the different generations of cells it is found to exhibit

throughout essentially the same features as mark its history in *speciosus*. The only observable difference is in the size of the parts. One individual, however, exhibits a variation in appearance of the multiple, which makes the conditions in *Anabrus*, already described ('05), directly comparable to those of *Hesperotettix* (fig. 23, pl. 4). Instead of the accessory being attached to the end of a rod-shaped tetrad it is joined to one extension of a ring. This relation appears in the prophase and is carried over directly to the metaphase, so that multiple chromosomes of very different shape from the ones commonly present are produced. Both forms appear, not only in the same animal, but also in a single cyst. It is probable on this account that the variation does not represent any fundamental difference, but only a divergence in some rate of movement of the parts.

The spermatogonial complex shows practically the same conditions which characterize the corresponding stage in *H. speciosus*. Minor differences of relative size of the elements are the only ones of note.

Figure 23, plate 4 exhibits the complex in the first spermatocyte. It is at once noticeable that the chromosomes are strongly of the *Hippiscus* type and in polar view appear as rings or V's lying in the equatorial plate. The only unusual feature is the tetrad, elongated in a plane of the spindle axis, with the accessory chromosome joined to one end at a more or less acute angle. The behavior of the chromosomes during division presents no unusual features and my general description of these types of chromosomes, given at length in a former paper ('14) may be held to apply here. The second spermatocyte mitosis likewise is typical for the euchromosomes, and the multiple chromosome behaves like the same structure in *speciosus*.

#### 4. *The complex of H. brevipennis*

There is nothing in the specimens of this species, so far examined, to indicate the existence of any of the unusual conditions of chromosome association which occur in *speciosus*, *pratensis* and *viridis*. As may be seen by an examination of plate 3, figure

8, the complex is strictly of the *Hippiscus* type. There are neither multiple nor atelomitic<sup>1</sup> 1 chromosomes. It is possible that a more extensive series might show some variation, but this is doubtful. If multiple chromosomes are characteristic of the genus—which might fairly be said from their prevalence in the species so far studied—then *brevipennis* departs here from the type. Since this paper deals only with the question of multiple chromosomes, no further consideration will be given to species in which they are absent, except to note that the seriation in size is uniform.

#### 5. *The complex of H. festivus*

This striking and characteristic species, of which numerous specimens have been studied, is like *brevipennis* and shows no trace of multiple chromosomes. The nature of the complex in the first spermatocyte may be observed in plate 1, complex 2. It seems therefore to have no immediate bearing upon the subject of the present discussion.

#### 6. *The complex of H. viridis*

This species, like *speciosus* and *pratensis*, may possess a multiple chromosome, a hexad, constituted of a tetrad joined to the accessory chromosome dyad. The euchromosome portion is larger than those of the other species. This was the condition present in the first specimens which I studied and which served

<sup>1</sup> *H. viridis*. In a former paper I found it convenient to refer to various forms of chromosomes and to note that their forms depend upon the fiber attachment. Following de Sinety, the position of this attachment was designated as terminal, subterminal, or median. In the case of the annular chromosomes terminal attachment apparently results in rings whose plane in metaphase coincides with that of the equatorial plate, while with subterminal, or median, attachment the rings are so placed as to lie parallel with the spindle axis. For convenience these were spoken of as the *Hippiscus* type and the *Stenobothrus* type respectively. With the discovery of additional complexity of forms it has been found desirable to have general terms for the two conditions represented by the ring chromosome, and so it has been agreed in this laboratory to speak of chromosomes with terminal fiber attachment as 'telomitic,' while those with non-terminal attachment receive the name of 'atelomitic.' These terms may therefore be used to designate chromosome conditions similar to, but more general than, the ones involved in the two types of ring formation.

as the basis for my earlier discussion, although at the time they were not fully identified individuals. Now, with an extensive series secured for me by Miss Carothers, I find that the uniformity of association, so marked a feature of my early slides, has, for certain chromosomes, given way to an order characterized by multiples or non-multiples in equal proportions for the individuals studied. For any individual animal however there is no variation. But this species possesses added interest because of associations between euchromosomes to produce octad multiples in which the accessory chromosome does not occur. This is the first unquestionable instance of any such condition in the Acrididae, but its occurrence in a genus of the Locustidae has recently been reported by Miss Woolsey, working under the direction of Robertson. So clear and unequivocal is this condition in viridis that its interpretation can not be escaped, and that it should have failed to appear in my early material is most surprising. A careful restudy of my preparations makes it evident that no other multiples than those involving the accessory chromosome are to be found there. In some more recently prepared slides of earlier lots I was however somewhat chagrined to find individuals with the accessory chromosome free. These various modifications of the complex are most interesting and suggestive and merit extended consideration.

The following conditions of the first spermatocyte complex have been encountered so far (plates 1 and 2).

Class (1) -12 separate chromosomes = 11 tetrads + the accessory dyad

Class (2) -11 separate chromosomes = 10 tetrads + one hexad

Class (3) -10 separate chromosomes = 8 tetrads + one octad + one hexad

Class (4) - 9 separate chromosomes = 6 tetrads + two octads + one hexad

Class (5) -10 separate chromosomes = 7 tetrads + two octads + one dyad

Class (5a) -11 separate chromosomes = 7 tetrads + two octads + one dyad  
and supernumerary

Class (6) -11 separate chromosomes = 9 tetrads + one octad + one dyad

While the number of chromosomes varies from nine to twelve, the number of chromatids, morphologically recognizable, remain in all cases forty-six. The particular form of chromosome which I called an 'octad' in my former study ('05) presents itself very commonly in the new material.



A discussion of these various classes of complexes will serve to bring out most clearly the conditions prevailing in the species. Of these the first shows no unusual conditions whatever, and had specimens of this nature been the only representatives of the genus examined, they would have been regarded as coming from a group entirely typical of the family. There are the usual twelve chromosomes in the form of rings, V's, crosses and rods as found in *Hippiscus*, all of the telomitic type. The accessory chromosome is free and early passes undivided to one pole of the spindle. Of thirty-eight individuals studied, five had this chromosome constitution.

Seven of the thirty-eight belong to the second class and were the only kind of which I had preparations when my first study was made. Conditions here are as in class 1, with the striking exception that the accessory chromosome is joined permanently to one of the tetrads, forming a hexad element. The structure and behavior of this complex of eleven chromosomes instead of twelve, has been fully described and will not need further consideration here.

Very different is the aspect of the complex in the five individuals in class 3. Instead of twelve separate chromosomes as in class 1, or eleven like class 2 there are but ten. We recognize at once the hexad multiple of the second class and, in addition, eight telomitic tetrads of characteristic forms. The tenth element is much the largest and is otherwise strikingly different from its mates. In shape it is a much elongated ring of the *Stenobothrus* type, but, like the rings of *Chloealtis*, usually lacking any lateral extensions. Not infrequently there will be a separation on one side of the ring producing a C-shaped structure. The character of this element will be considered later in connection with other chromosome forms.

Members of class 4, of which I have seven individuals, show still further modifications. The number of chromosomes is reduced to nine, among which are distinguishable six tetrads of usual types, two large *Stenobothrus* rings and a hexad multiple. The two differential rings are of unequal size. The larger is directly comparable to the similar element of class 3, but the

smaller is always recognizable by its minor size and also by its variability of form. In this latter respect it may appear as a fully formed ring, or it may show varying degrees of separation at one of the polar angles, resulting in a large V-shaped structure. Any one individual animal however shows either a ring or a V and this does not suffer variation in any cells.

In class 5, represented by seven individuals, we encounter again the number ten as in class 3, but it is constituted in a different manner. The absence of the hexad multiple is at once apparent, for the accessory chromosome is free and occupies a characteristic polar position. Its sometimes associated tetrad appears among the seven chromosomes of this type and there are in addition, two large rings as in cells of class 4. So far as can be seen, these two elements are as directly comparable with the corresponding two of the preceding class as are similar structures within either of these classes.

To the complexity resulting from combinations of separate elements into multiples, thus reducing the number of independent structures, there is added the opposite condition of a supernumerary chromosome in the animal found in class 5-a. This has a complex similar in its composition to the ones in class five to which is added a small, extra element with the usual characteristics of the supernumerary chromosomes. For the third time there appears the number eleven, but its make-up is different from the complexes of either class 2 or class 6.

The number eleven reappears in cells of the six individuals in class 6, but once again there is lacking the hexad multiple characteristic of classes 2, 3, and 4. Nine ordinary tetrads, a free accessory chromosome and one large *Stenobothrus* ring make up the complex in this group. In place of the smaller ring, or V, of classes 4 and 5 there are present two rod-shaped chromosomes of corresponding size. The large ring occurs in five of the six individuals and is directly comparable to the element falling in the same place in complexes of classes 3, 4 and 5. The sixth specimen of this group has, in place of the ring, a large V of the same construction as the smaller octad in class 4. This is the only individual in which the larger tetrads were not united at

both extremities and, until it appeared, I was inclined to believe that the unipolar union, rather common in the case of tetrads nine and ten, was lacking and that some modification of the association force existed. It is now evident that the conditions are essentially the same in both cases, except that the more frequent occurrence of the large ring and its complete character would suggest a longer history of multiple constitution.

### III. CHROMOSOME CONDITIONS IN THE GENUS *MERMIRIA*

At the time of my first report ('05) upon *Mermiria* I had studied all the available Acrididae species and, in every individual, found only one type of chromosome—a telomitic rod in the spermatogonium, which, after synapsis with its mate, appeared in the form of a Hippiscus-type ring or in some modification of the type. Although familiar with the atelomitic form of chromosomes in other material, I had never seen it in the Orthoptera and had no reason to suspect its occasional appearance. Failure to consider the possibility of such chromosome forms was responsible for my misinterpretation of the multiple chromosomes in *Mermiria*, through which I made the serious error of reporting the segregation of whole tetrads. Although, with increased knowledge of the conditions in the group, it is now possible to determine with certainty the nature of the multiple chromosomes, at the time of my first acquaintance with the phenomenon of chromosome combinations the constancy of chromosome structure and behavior was so marked as to make quite unjustifiable any assumption of variation. Added to this is the fact, which seemed at the time very significant and which even yet is not satisfactorily explainable, that in certain individuals the multiple has a series of definite constrictions corresponding in number and position to what would exist in a decadal element. Finally; actual separation in the first spermatocyte metaphase at the level of these constrictions in certain cells persuaded me of their value as an indication of chromosome boundaries. The theoretical difficulties involved in explaining the preservation of the complex on the assumption of tetrad segregation finally convinced me that I was probably mistaken, but

it was not until the discovery of the J-shaped chromosome in *Trimerotropis* by Miss Carothers that any explanation, consistent with the other known conditions in the group, was made possible. Meanwhile I had accumulated an extensive series of specimens and had studied the complex of the female and needed only the conception of the J-shaped tetrad to bring all the observations—with the exception just mentioned—into conformity.

In *Mermiria*, as in *Hesperotettix*, the fixity of combinations which seemed to mark the genus has failed to prevail throughout all its species. While such variation as is shown by *H. viridis* does not obtain in any species of *Mermiria* studied, multiples are lacking in *texana* and *neomexicana*. These results emphasize strongly the necessity of an extensive series of specimens in any investigation—a requisite I have always appreciated and sought to meet. Our recent work in the Orthoptera has shown that numbers of considerable magnitude are required for extensive generalizations.

#### *Mermiria bivittata*

It is not my purpose in the present paper to enter into a comparative history of the chromosomes in the various species of *Mermiria*. I wish merely to consider the multiple chromosome found in *bivittata*, with its modifications in certain groups of individuals which seem to have specific value. My former report on this species stated that the multiple chromosome consisted of two tetrads joined to the accessory chromosome. This is a mistake the occasion for which is discussed elsewhere. More extended study upon a large series of specimens has shown that in this genus, as in *Hesperotettix*, there is a hexad multiple instead of a decad. While there is a striking difference in configuration of the element in the two groups, it is due entirely to the form of tetrad involved. In *Hesperotettix* this is an ordinary extended rod with the accessory chromosome joined at approximately a right angle on one end. *Mermiria* has, on the contrary, a tetrad already of just this shape, to the straight end of which the accessory chromosome unites. Thus, while the composition is the same, the appearance is very unlike. How



this configuration is established, appears very definitely when the chromosome complex of the different cell generations is studied.

Conditions of the spermatogonial metaphase are presented in figures 44, 46, 47, 49, pl. 6. First it may be noted that the number of chromosomes present is twenty-two instead of the usual twenty-three, and that, of these, two differ from the remainder and from the usual spermatogonial rods in being atelomitic V's, with more or less unequal arms. Even a casual study of these differential elements reveals their divergence in both size and structure. One is distinctly larger, and, under favorable conditions, it may clearly be seen that its shorter arm is irregular in outline and more lightly staining. In these respects it conforms to the behavior of the accessory chromosome in many Orthoptera. So characteristic are these appearances that there can be no doubt whatever in the identification of this arm of the V chromosome as the accessory. The V chromosome, then, is a multiple, a tetrad, consisting of the two rod-shaped chromatids of the accessory chromosome joined to the two corresponding rods of a euchromosome, just as in *Hesperotettix*. At the angle of the V, where the fiber is attached and where the chromosomes join, there is, not infrequently, a clear break in outline. The compound nature of this V is clearly evident from these facts.

Inspection of the smaller V reveals no such indications of heterogeneity, and only in shape does it differ from the remaining elements of the complex. Its position in the metaphase plate is commonly near the multiple V. For convenience of comparison several of these pairs of V's in different stages are shown in figures 47 and 49. The number of chromosomes present in the complex is therefore found to be twenty free telomitic rods of various lengths, two rod chromosomes joined at their inner extremities forming a V and one other V-shaped chromosome—a total of twenty-three, the number characteristic of the family. The internal morphological evidence of the complex in this generation is sufficient proof to establish this conclusion. In addition however we have two confirmatory lines of evidence which are of great value, viz., the subsequent history of these structures in spermatogenesis and the conditions of the female



diploid complex. Before following out the later spermatogenetic history I will briefly indicate the conditions in the female cell.

Unfortunately it has not been possible to work out the history of the female germ cells, but the egg follicle cells show beautiful, clear chromosome groups, some of which are represented in figures 43, 45 and 48. It is at once observable that the resemblance to the male diploid group is very marked. Here again there are twenty-two chromosomes, among which are also two V's. A more careful study however reveals a significant sexual difference in the case of the two V's. Instead of being unlike in size they are of practically identical proportions. Owing to their somewhat sinuous course through the cell, with consequent foreshortening, it is difficult, if not impossible, to represent them accurately in drawings. Where they lie more nearly in the same plane, as in figure 43, pl. 6, their equivalence is clearer. Under the microscope there is no difficulty in appreciating the close resemblance existing. On comparing these two V's with the ones of the spermatogonium (figs. 47, 49, pl. 6) it is seen at once that in size and proportion they agree with the larger one of the male cells. In other words these are two multiples, the short arms of which are the accessory chromosomes. The count for the female complex, instead of the apparent twenty-two, is therefore twenty-four, corresponding to the conditions in other species. No differential behavior of the sex chromosomes was observed in these female somatic cells and but little evidence of separation at the point of fusion.

Other species of *Mermiria* show no multiple chromosomes in the first spermatocyte, and when the spermatogonial complex is observed there are found twenty-three rod shaped chromosomes of the usual type. Absence of a multiple chromosome in the spermatocyte is accompanied by the absence of V-shaped chromosomes in the spermatogonium. Animals of this type are not classified as *bivittata*, but, quite aside from their exact taxonomic disposition, it is plain that they must be very nearly related to *bivittata*. In such material, then, the direct relation between V chromosomes of the spermatogonium and multiple chromosomes of hexad nature in the first spermatocyte is

strongly suggested. Because of the constancy of chromosome organization, indicated among other ways by the constancy of fiber attachment, we would be justified in saying that the V's of the spermatogonium unite in synapsis to form the first spermatocyte multiple and that this should appear with two non-terminal fiber attachments. Such a condition is realized in the structure of the hexad in the first spermatocyte metaphase.

The conditions of the first spermatocyte complex in metaphase are readily determined, particularly if sections of sufficient thickness are used, and in smears are almost diagrammatic. Here there are clearly eleven chromosomes present, among which is a very large and distinctly different shaped one (figs. 55, 56, 57, pl. 7). This is characteristically in the form of a rod with the two ends of slightly different length bent sharply back in the same plane. One or both of the ends may exceed, or fail to reach, the common angle, producing some variety in form. Greater or less extension, preparatory to division, may result in considerable variation in length, but commensurate and opposite changes in diameter show that the volume remains very constant. Indications of internal composition are afforded by the contour of the element, although, as will be shown later, they are not entirely trustworthy. At each of the bends, where the fibers attach, there is a constriction, and nearer the shorter bent end, at about its length down the shaft, there is a pronounced fissure.

At the time of division, separation occurs at this point, producing two unequal V's in the anaphase. Although the chromosomes are much shorter and thicker than in the spermatogonium, relative proportions are preserved, and if these two parts of the long chromosome (figs. 47a, 50a, pl. 6) be compared with the two V's of the spermatogonium they will be found to correspond almost exactly. That is to say, two V-shaped chromosomes of certain proportions found in the spermatogonium reappear in the first spermatocyte (united by one limb) and are there separated at this point and segregated into different second spermatocytes. Since one limb of one V is the accessory chromosome, it remains undivided, as usual. Attached to the accessory chromosome is the rod portion of a J-shaped chromosome which

has separated from its V-homologue to which it was joined in synapsis. The only differences between *Mermiria* and most other Orthoptera are (a) the multiple chromosome V and (b) the euchromosome V. That the union of the accessory to a euchromosome is not a fundamental change of the nuclear state is indicated by the fact that certain species of *Mermiria* lack the association. No change in the distribution of the accessory results from its union, and, as we have seen in *Hesperotettix viridis*, the combination may be so weak as not to occur in some individuals.

Evidence that the euchromosome V is of like transitional character is not wanting in certain well marked members of this loosely constituted species. In these (figs. 58, 59, pl. 7) the multiple chromosome of the first spermatocyte metaphase is much like that of *Hesperotettix* except in proportions. There is a pronounced bend at one end at the point of fiber attachment, but the other extremity is almost straight, only a slight subterminal flexure indicating the place of the other fiber insertion. In some instances this point is almost at the end of the chromosome. Individuals with this peculiarity are clearly distinguishable by somatic characters and, I believe, constitute a distinct species. It is possible that, with fuller representation, forms similar in the constitution of their multiple chromosomes to those of *Hesperotettix* might appear.

This type of hexad caused me much difficulty and led to the conception of a decad chromosome. As will be noticed in figures 58 and 59, pl. 7, there are a number of constrictions along the length of the chromosome quite constant in position, and so placed as to indicate that there are five divisions or parts. It will also be observed that the chromosome may be divided at more than one of these levels. I can not now find any explanation for these separations at various levels on the chromosome, but that they are evidences of unions between various tetrads is not indicated by the full history of the chromosome complex. Again, in the anaphase and telophase of the first spermatocyte, the composite nature of the larger V becomes marked. As may be seen in figure 13 of a former paper ('05), one member of the

V consists of two granular rods of the same character as the remaining chromatin elements, while the other arm is composed of two dense and homogeneous rods of smooth contour. All these parts tend to diverge widely except at the level where the unlike portions join, which is the point of fiber attachment in the preceding cell generations and its site in the one to follow. This difference in constitution is the reverse of the one in the spermatogonium, but corresponds to the relative degree of concentration of the nuclear elements in the first spermatocyte prophase. The interval between the two spermatocyte mitoses is very brief and the chromosomes may be followed through their changes without any loss of identity.

The metaphases of all second spermatocytes show eleven separate chromosomes of which one is a V. On observing these V-chromosomes (figs. 63, 64, pl. 7), however, it is found that they are of two sizes which correspond in proportion to the two V's of the spermatogonium and to the two of the first spermatocyte anaphase. Upon division these are distributed to the spermatids which are accordingly of two classes, equal in number. It is clear from this very evident history of the two V-chromosomes that they go into different spermatozoa and so, upon fertilization, are contributed to different individuals. Of some theoretical interest is the fact that the rod-shaped homologue joined to the accessory chromosome has a criss-cross inheritance while its V-mate is confined to the male line. The presence of the accessory chromosome as a portion of one V means the addition of one chromosome to the count of eleven separate elements, and so conforms to the conditions in other Orthopteran species.

#### IV. GENERAL CONSIDERATIONS

The very extensive and detailed studies of chromosomes which have been in progress for many years would not be fully justified if the result were merely a record of interesting but non-significant protoplasmic manoeuvres. It is the belief that the substances of the chromosomes are specific materials which are intimately concerned with the development of a multitude of



dissimilar cells from a single cell that renders a knowledge of the finest details of their structure and behavior of the utmost importance. That this belief is well founded would seem to be clearly indicated by cumulative evidence from many sources. The fundamental concept involved is the Roux-Weismann hypothesis that the chromatin is the idioplasm, which is differentially organized and linearly arranged. As an indication of this differential organization and linear arrangement, the existence of definite aggregates of the chromatin substance into more or less thread-like chromosomes is regarded as most important.

Details of chromosome structure and behavior are significant, therefore, as indexes of the precision of organization in the material of which they are composed. Since it is the existence and perpetuation of this definite series of differentiated materials that is primarily required by the hypothesis, it is conceivable, and possible, that it may vary in the nature of its aggregates into definite masses (chromosomes) without affecting the manifestation of its various specific effects, except in their combinations. It is not the existence of a certain number of these aggregates that is of first importance, but the presence of the varied materials which enter into their composition. While this is true, it is to be expected that a somewhat exact correspondence should be maintained between the ultimate units of differentiated substances and the units of higher order into which they are assembled. But such a fundamental arrangement may be maintained in the presence of both lower and higher numbers of chromosomes through secondary combinations into units of still higher value in one case, or through duplications of the normal series in whole or in part, in the other. Numerical variation is not of itself *prima facie* evidence of altered organization—it must be shown that something of the complete series is lost, or new and unrepresented materials added, in order to demonstrate the existence of altered organization. The maintenance of the morphologically recognizable units of the original series in the face of changed conditions is indeed added proof of the exactness and stability of structural conditions in the chromatin substance. Evidence of a very important character in support of this position is fur-



nished by the facts disclosed in the study of *Hesperotettix* and *Mermiria*. For convenience these facts may be taken up under a number of different headings.

### *1. Chromosome numbers in the Acrididae*

It is claimed by those who criticise the so-called chromosome theory of heredity that the maintenance of the specific number of chromosomes is required and that variations in number are direct disproof of the theory. There is an element of truth in this argument which has always been granted by students of cellular phenomena, but stated baldly and without reservation it may lead to entirely erroneous conclusions—indeed has done so in conspicuous instances. The fallacy in the argument lies in the circumstance that a primary organization may be maintained while having superimposed upon it secondary modifications. Instances of this are very common in organic structures. It is no argument against the reality of the pentadactyl type of limb that duplications or combinations or reductions occur in certain groups or individuals. Polydactyl or syndactyl individuals reproducing their modifications of the primitive type are not regarded as illustrations of the absence of a fundamental organization of limb bones. The diplopod condition is rightly considered as secondary to the usual arthropod arrangement—not a disproof of its existence. While it is true that these instances of organization are of a different order from those found in cellular structures they are true examples of meristic variation in individuals in the presence of a persistent type. All that is claimed by those who believe in the hereditary significance of nuclear structures is that the chromosomes are, in themselves, indications of structural organization in the materials of which they are composed. Provided the full complex of elements be preserved, the essentials of idioplasmic control of development exist, even in the presence of combinations or duplications. From this point of view, in the event of apparent variation in chromosome numbers in an individual or group, it is essential to discover whether any of the normal chromatic units are lost or others of different

nature added before it can be established that there is not maintained a specific organization.

The belief in such an organization as this has, for brevity, been called the theory of chromosome individuality. This has perversely been much misunderstood or misrepresented. According to some the theory demands an 'independent existence' of the chromosomes, although how this could seriously be maintained in the entire absence of any independently existing chromosome or claim for such, it is difficult to see. In the minds of others the theory demands that the chromosome shall 'make the cell'—whatever that may mean. Of course no such views are entertained by any cytologist and no fair interpretation of the theory of chromosome individuality would give occasion for such statements. Because it is desired to examine the nature of the evidence in favor of this theory, under conditions which might apparently controvert it, a statement of the facts, involved and an outline of the theory may be given to avoid useless discussion. They might take this form in the present state of our knowledge (1) The cell is a complex of organs having various functions; (2) of these the nucleus, is most concerned in reproducing the characters of the cellular organization; (3) of nuclear substances those involved in the structure of the chromatin are most important in carrying out this function; (4) these substances are likewise differentiated and have various rôles; (5) an indication of this differentiation is afforded by the aggregation of these substances into certain definite masses which are characterized by individual peculiarities of size, form and behavior; (6) these aggregates maintain their organization and reproduce themselves in each cell division; (7) their derivatives are characterized by similar attributes of size, form and behavior under given conditions; (8) such complexes therefore occur in all the cells of an individual, both germ and somatic; (9) because members of a species are related by descent their complexes are essentially the same; (10) the fact that a type of organization, which holds invariably for the individual and almost as constantly for the species, prevails with slight variations through genera and even a family is very strong evidence for exactness of organization:

(11) the behavior of the chromosome in maturation and fertilization is strictly in accord with the necessary mechanism for alternative inheritance; (12) the inner constitution of the chromosome is such as to afford an explanation for the linear arrangement of factors and for their variations; (13) in the instance of the most thorough analysis of characters in any one animal, *Drosophila*, the groups of linked characters and their magnitudes correspond to the number and size of the chromosome; (14) experimental disturbance of chromosome conditions is followed by the expected modifications of characters during development; (15) from the known conditions of nuclear organization in relation to character development predictions with regard to new characters and combinations of characters may be made.

As has been stated before, the number of diploid chromosomes in the various species of Acrididae is usually twenty-three in the male. Exceptions to this have been announced by Granata ('10) for *Pamphagus* and by various authors for *Chorthippus*, and by myself for *Hesperotettix* and *Mermiria*. Montgomery gave the number twenty for *Syrbula acuticornis*, but this is unquestionably an error, as Robertson has shown. In no case has a variation been reported for cells of the individual. The gametic complex therefore maintains itself, according to the reports of all investigators. Similar constancy prevails in the species, in almost all instances, except for the wide variation of eighteen to twenty-three here reported for *Hesperotettix viridis*. In the face of the admitted validity of numerical constancy as one test of the theory of chromosome individuality, how can it be upheld when actual variations of this magnitude exist? This would seem to be as severe a strain as it could be subjected to, and if it can be shown that the conditions in *H. viridis* are capable of explanation without invalidating the hypothesis, then other cases of apparent exceptions would be less weighty as evidence until their full character became known beyond question.

On first thought the conditions in this group would seem to be a particularly strong argument against the theory. Here is a species, beyond question intimately related to many others of a

group in which the number of chromosomes is almost constant, and yet, within the one species, there are wide departures from the normal number. If such changes may occur without producing any effect upon bodily characters that will serve to mark individuals by their variations, then surely it may be argued that there can be no direct relation between chromosomes and somatic structures. This would indeed be a vital objection to the theory if, under its terms, the existence of a fixed group of free chromosomes of unchanging behavior were postulated. Such however are not the conditions of the theory. If it can be shown that the smaller numbers present in some individual are not caused by the loss of any chromatic units, and further that all the conditions in the group are consistent with the maintenance of certain associations between chromosomes and their chance combinations in fertilization, then the conditions of the theory are not violated. In effect this position would extend the theory beyond the observed conditions of the chromosomes to their subdivisions. It would be most exact if it could be based upon constancy of chromomeres, the limit of our observational analysis, as has been done for certain chromosomes in *Phrynotettix* by Wenrich ('16).

The genus *Hesperotettix*, so far as our studies have gone, has two species, *brevipennis* and *festivus*, in which the normal haploid number in the male is twelve; two species, *speciosus* and *pratensis*, in which the number is eleven; and one, *viridis*, in which both of these numbers are represented and, in addition, nine, ten and thirteen. There can be little doubt that the occurrence of the typical family number in the genus is significant of conformity to type; the presence within one species of this number, and of variations from it, most strongly indicates that, whatever differences there are, they must not be due to any fundamental disturbance of the chromosome organization. This *a priori* argument, while in no sense conclusive, has its value and must be considered. Fortunately however there is strong objective evidence to support it.

First of this evidence is the case where the number is reduced, from twelve to eleven. This I have already considered at length



('02, '05), and the case is supported by observations of Sinety ('01) and others. In this instance an element, so well marked by peculiarities of form and behavior as to be unmistakable in every cell generation, is found to be united to one of the euchromosomes at all times so as permanently to reduce the haploid number to eleven. There is actually one less free and independent chromosome in cells of these animals than in those of other members of the group. This however is a far different thing from saying that there is one less chromosome present. In fact there is no difference at all in the number of chromosomes—the sole variation being the union of the accessory chromosome with one euchromosome. To put the case thus is to make a plain statement of fact and not to create an hypothesis. The apparently missing element is just as clearly present as in any other Orthopteran cells. Irrefutable proof is thus afforded of the process of the fusion of chromosomes without loss of their identity. As a principle of nuclear organization we have therefore to reckon with the ability of chromosomes to unite together while still retaining their morphological integrity.

It is also conceivable that where a tendency of this kind manifests itself between certain chromosomes in a species, it may also obtain between other members of the complex. Such combinations are more difficult to detect on internal structural evidence, because there is no such differential behavior as characterizes the accessory chromosome. Nevertheless there are valid criteria which may be employed and these make certain the existence of such multiple chromosomes. The conditions displayed in *H. viridis* are peculiarly fortunate and may now be considered in detail. From the study of thirty-eight individuals of *H. viridis* it is apparent that (a) the number of chromosomes in the first spermatocyte may vary from nine to thirteen and that (b) the same number may be present in two or three individuals and yet in each case be differently constituted. The necessity for a careful and detailed study of the complex before passing judgment upon the significance of numerical variation, is certainly apparent from these circumstances. In view of the fact that most explanations of changes in the number of chromosomes have concerned the smaller members of the series, it is of



much interest to find that in *Hesperotettix* this portion is relatively stable while the larger elements may be variously combined together. But however much difference there may be in numbers there is apparent no loss of any morphological structures in any of the classes. A comparison of homologous series of chromosomes, based on size, may be made on inspection of plate 2 where I have attempted to arrange the chromosomes of different classes in order so that homologous chromosomes are in vertical rows. In the case of multiple euchromosomes they are placed in an intermediate position between the columns where their members would properly come. The accessory chromosome, when in a multiple, is drawn as it is attached to its tetrad.

Even a casual inspection of this plate will show that, aside from the accessory chromosome, the seven smaller chromosomes, (nos. 1 to 8) constitute a graded series in which variation is not great. The eighth chromosome, however, may or may not have the accessory chromosome attached to form a multiple. In classes 4 and 5 there are above this chromosome only two other separate ones, but these are of a character to attract attention at once. Unlike most of the remaining elements of the complex, they lie extended, parallel with the spindle axis and are in the form of open rings, each half of which is greater than the whole of chromosome eight. There are a number of considerations which make it practically certain that these are multiples composed respectively of chromosomes 9 and 10, 11 and 12.

First, it is to be noted that if these are counted as octad multiples, the number of chromosomes becomes exactly twelve. In weighing the value of this piece of evidence it must be remembered that this number is characteristic of almost the entire family to which *Hesperotettix* belongs. This fact makes it certain that in this respect we deal with such a fundamental feature of organization as to require us to consider any variation, not as *prima facie* evidence of a lack of precision in organization, but rather as a modification of the form of it.<sup>2</sup> In the

<sup>2</sup> One of the most unjustifiable and unscientific attitudes imaginable is that which would regard the effort to interpret the form of organization in its various aspects only as an attempt to force a real divergence into a seeming agreement. Such a style of argument has been resorted to by a number of those whose belief

case before us the individual animals in class 1 have twelve chromosomes, those in class 4 have nine while the ones in class 5 have ten. The question we have to decide is, whether these conditions represent absolute differences in organization, or whether, within the nine and ten groups, the twelve elements of class 1 are morphologically present. On inspecting a complex of class 4 we find that the accessory chromosome is missing from its usual position in the series, but upon looking further it is discovered, with all its usual characteristics of structure and behavior, attached to one of the tetrads. As a structural element of the cell it is demonstrably present and must be counted, although it is not a free chromosome. In a complex which is of almost identical composition otherwise, (class 5) the accessory chromosome is a free element and would of course be counted. In both cases the entire history of the accessory chromosome in all cell generations is typical, whether free or attached. The remaining two chromosomes of classes 4 and 5 are strikingly different from all the others, and upon careful inspection are found to have morphological subdivisions, which if counted in the usual way, restore the apparently missing two. In other words, if the number of chromatids is counted the total is forty-six, just as it is in class 1. So far as the number of elements is concerned, therefore, we are dealing with the same series in each case. It is obvious that if the differences in number were the result of an unordered variation there would be every reason to expect numbers in excess of twelve, together with modifications of all the elements and not of precise changes in particular ones.

In a similar way, when the numbers in classes 2, 3 and 6 are considered, the same evidences of ordered change are seen. Only eleven separate chromosomes are found in class 2 but the largest of these shows very clearly the accessory chromosome as a con-

is that the chromosomes are unstable and indefinite structures of the cell. Back of such a position is the implication that evidence must be taken at its face value without interpretation. This I consider entirely wrong. The essence of observational work is interpretation, and in microscopical investigations it reaches its fullest development. The value of evidence submitted by a microscopist is in direct proportion to the quality of his interpretative ability.

stituent part and thus demonstrates the completeness of the series. In class 3 there are ten separate chromosomes, of which one is a multiple involving the accessory chromosome as in class 2, besides which there is a very large octad similar to the one in classes 4, 5, and 6. Class 6 differs from 3 only in respect to the non-union of the accessory chromosome with a tetrad. The one individual of class 5 a, of which I have only a smear preparation, presents the second instance of a supernumerary chromosome which has appeared in my material up to the present. Aside from this, the complex is like that of class 5. From all these considerations I think it may be said that the numerical variations within these specimens of *H. viridis*, instead of being an indication of lack of stability in organization of the chromosomes, are, in fact, very strong proof to the contrary. There is nothing whatever to indicate that the integrity of any element is lost, or, aside from the case of the supernumerary, that any additional members are added to the complex. Variation is not a question of loss or gain, but of relations.

## *2. Chromosome numbers in general*

Variations in chromosome numbers, within the species and larger groups certainly exist, as is clearly manifest in the work of numerous investigators. The constancy of numbers is more definitely established in some groups of animals than in others. Thus the Acrididae have a common number for many of the genera, but in the Hemiptera, as Wilson and Montgomery have indicated, the families are much less uniform numerically. In using the conditions of the family which I have most studied as an indication of chromatin organization, I have recognized that the nature of this organization might be different in other groups, and I have not attempted to apply generally the immediate conclusions to which I inclined. Not only is this true but the specific statement to that effect appears in an early paper of mine ('08 a) in these words:

I am quite prepared to admit also that in one species even, there may be a variation in the integration of the chromatin material, resulting in some numerical variation of the chromosomes, without losing

my belief in the necessity for this definiteness in the grasshoppers. We do not yet know how much difference there may be in the organization of the various chromosomes of a complex nor how variable in importance they may be.

A more careful reading of my papers would have saved some of my critics much futile argument.

While I have thus avoided any consideration of the general topic of chromosome variations in other papers, I should like now to examine some of the data relating to this subject. It is important that this be done, because most of the attacks upon the theory of chromosome individuality have proceeded from investigators who either found, or think they found, variations in chromosome numbers within individuals or species. Such attacks have run the gamut, from assertions that chromosomes are merely physical aggregates without morphological value, down to questions regarding the definition of the term chromosome. In undertaking such a general discussion I shall base my opinion largely upon material with which I have personal familiarity. Much harm has come from attempts to homologize results from widely different materials by persons who have no first hand acquaintance with the conditions discussed. With our present knowledge of cellular phenomena as slight as it is, such lengthy critiques, involving the interpretation of other investigators' interpretations, can accomplish little good and may greatly retard progress by fixing attention upon relatively unimportant details. A striking instance of this has been the controversy regarding pre- or post-reduction, extended long past the time when it was definitely known that the question of the segregation division is one of the individual chromosome and not of a whole mitosis. The matter of chromosome organization is, however, fundamental to all our present conceptions of the cell in relation to the larger problems of biology, and the evidence is cumulative that the essential assumptions of our hypotheses are justified. On the other hand it seems clear that the nature of chromosome integration varies with different groups and that, for this reason, we must be cautious in carrying over the conclusions reached from a study of one population of organisms to



another. It is in full realization of this need for care in generalization that I wish to examine the conclusions of others regarding chromosome numbers in the light of my own and my students' studies.

In making such an examination I can profitably take up only the most general questions, because, in matters of detail, it becomes a case of interpretation of phenomena, and many times one is not justified in criticising the work of others without himself knowing the objective conditions. For this reason I should like to consider the work of Della Valle on the subject of chromosome numbers. I do not choose this author as representative, because of any inherent strength apparent in his papers, nor am I impressed by their number and size. On the contrary they appear to me weak, because of the small amount of observational basis and the large development of theory. For instance in the paper of 1909 of the hundred and seventy-seven pages, only forty pages are included under the heading "Data of observation" and, of these, twenty are concerned with matters of technique. Throughout all of his publications, indeed, there is apparent a painful lack of judgment regarding the distinction between fact and theory, and were he alone in his position he could fairly be dismissed with little attention.<sup>3</sup>

<sup>3</sup> 'Objective analyses,' accomplished by the translation of morphological facts into terms of physical chemistry, carry their own indictment of lacking experience and judgment, and could safely be left without comment. Such an attitude toward chromosome organization is, however, but an extreme case of the position assumed by a number of biologists who would seek to discredit the hard won facts of cytology by an appeal to conditions or forces beyond observational control or by an arrogation of the whole problem to their own chosen field. It would seem that, in almost every case where such attitudes are assumed, there is some animus or prejudice in the mind of the writer which betrays itself in his injudicious or intemperate language. All those who fail to agree with his particular extreme views are depicted as banded together for the establishment of dogma and the suppression of truth. With much vigorous language and the plentiful use of exclamation marks all such are consigned to scientific oblivion with their obsolete methods and narrow views.

This is very unfortunate. It is rarely the case that scientific investigators are not honestly in search of the truth with regard to the subject of their enquiry, and to accuse them of conspiring for its suppression is most absurd. It is true that new views sometimes prevail but slowly, but it does not hasten their



The work of Della Valle is open to criticism from two sides. It may in the first place, well be questioned whether the knowledge of colloid chemistry, and of fluid crystals in particular, is sufficiently established to justify the extension of its principles into the operation of cellular phenomena. Certainly one is inclined to doubt the basis on which such application is made when the complicated phenomena of chromosome division are declared to be 'absolutely identical' with the cleavage of fluid crystals, or when the involved changes of the chromatin in the telophase are described as 'identical' with the solution phenomena of a gelatin cylinder in warm water. It is of course not to be doubted that in the activities of the chromosomes, and all parts of the cell indeed, chemical and physical laws are operative. Such a belief is however far removed from the one which conceives some particular manifestation of chemical or physical energy as 'identical' with the behavior of the chromosomes in mitosis. Much might properly be said with regard to this phase of Della Valle's work, but I desire rather to consider the nature of what he advances as direct evidence against the theory of chromosome individuality. Since this involves a thoroughgoing denial of all the facts and theories relating to the subject, such a discussion will touch upon most of the objections that have been raised by other critics and will avoid the necessity for repeating arguments involving only minor differences of material or opinion. A summary of his position with regard to matters relating to chromosome individuality follows:

He asserts that the number of chromosomes in a cell is due to the constancy in the amount of the chromatin and the median size of the single chromatin aggregates. This number suffers variation according to the law of fluctuating variations and is

acceptance to attack the motives of those who hold other opinions. A method does not commend itself as an instrument of value merely because it is termed an 'objective analysis' when it is obviously the application of a series of analogies. Neither does an observed fact cease to be such on being termed a 'hypothesis' or even a 'subhypothesis.' It is in fact not infrequently true that much of good in the work of certain biologists issues under a severe handicap because of the inherent evidence of poor judgment in their estimation of values in the work of others.

subject to conditions within the system of which the chromosomes are a part. The chromosomes are temporary and variable organizations of the chromatin, which form in the prophase and dissolve in the telophase. Variations in size of chromosomes are within the limits of observational error and are of the order of size variations in the droplets of an emulsion. Correspondence in size between chromosomes and nucleus finds its explanation in the physical condition of adsorption. The fact that chromosomes shorten proportionally to their original length shows they are homogeneous and indicates the identity of all the chromosomes in a mitosis. The previous history of the chromosomes in a line of cells can have no effect upon later generations because there is no continuity. Definite or specific organization is lacking and there is no perpetuation of a series through reproduction of individual chromosomes. All explanations of variations in number (called 'sub hypotheses') are declared to be untenable upon 'accurate examination' and certain determination of numbers in sections is stated to be impossible. As is customary with critics of this type, Della Valle, after decrying the spirit and purpose of those who do not agree with him, proceeds to attack their methods and declares that only his own technique yields infallible results. Sections can not give accurate enumerations of chromosomes, and only counts made upon stretched membranes are of value. Having thus put out of count the bulk of the work already done, he next makes a comparatively limited number of observations upon one type of material and arrives at the ultimate general conclusion that chromosomes are fluid crystals. With this solution of the problem accomplished, all minor questions are easily settled, because upon this major premise that chromosomes are fluid crystals and subject to all the laws known to pertain to such physical aggregates, certain variations of number, size, form and behavior must exist. I should like now to consider some of these assertions in the light of work known to me personally, first pointing out that all instances of order and system under varying conditions are direct evidence against his position.

The number of chromosomes within cells of an organism, he states, is variable, according to the law of fluctuating variations, because the number in any cell is due to the constancy in the amount of chromatin and the median size of the chromatin aggregates. Opposed to this is all the evidence, already given, regarding the high degree of regularity in the Acrididae where so many genera are alike in number, although the amount of chromatin varies widely. In this large group, the family, variation is infrequent, but in one species, *Hesperotettix viridis*, variation is common. Certainly under the terms of Della Valle's argument the greater variation should occur where there are the greater differences in amount of chromatin. But if there is any truth in the assumption, some variation would be expected in the cells of the individual and this does not occur in these Orthoptera. As reported for *Culex* by Whiting ('17) in the germ cells the diploid number is constantly six, Hance ('17) finds the same conditions generally true of the somatic cells, but Holt ('17), working upon the same material, discovers a range of variation extending from six to seventy-two in certain intestinal cells. It is very clear that the amount of chromatin is here not constant, while the median size of the chromosome remains practically unchanged. There is no correspondence, either, between the size of the nucleus, or of the cell, and the number of chromosomes. No evidences whatever of any balanced physical system, such as Della Valle advocates, appears in these cells of *Culex*.

Hertwig's outworn nuclear-plasma relation theory receives just as little support. In *H. viridis* the amount of chromatin is much the same in all the cells of a given generation and yet the number of chromosomes in the haploid condition ranges from nine to thirteen. Along with this fixity in the amount of the chromatin and variation in chromosome numbers, goes a constancy of size series up to certain chromosomes, beyond which there is a sudden change. The phenomena relate to no means or averages, but concern definite morphological entities. In place of fluctuating variations there is definite and determinable order. What happens is explainable, not upon circumstances of

chance relations between masses of substance in different 'phases,' but through a knowledge of the history of certain individually recognizable chromosomes traced through a large group of animals. Worthy of note is also the fact that in such cases as *H. viridis* the range of variation is strictly defined and falls within the limits set by the organization established in the family, except in the occasional instance of supernumerary chromosomes—two instances in the thirty-eight individuals studied.<sup>4</sup>

It is only upon the basis of entire identity of all chromosomes in a complex and of their temporary character that the Italian author has any argument at all. Building upon this he seizes upon every reported instance of difference of chromosome numbers as a support for his thesis. Every descriptive fact is labeled a 'sub hypothesis,' and so many of these are secured in this way as to convince him that the main hypothesis of chromosome individuality has no standing. Curiously enough, he conceives a great importance for the fact that one explanation for all the reported cases of numerical variation is not sufficient. On the contrary, he says, there are so many ways of accounting for such variation that there can be no constancy and no individuality. Perhaps in no other way is the quality of his argument better indicated than here. The fact that an error in observation has been made, and even admitted, is only an indication of variation; when it is shown that a reported difference in number is due to the inclusion of more than one species in a study this is a subhypothesis weakening the main one; the production, by hybridization, of chromosome numbers different from those of the parents is accounted as an example of variation, as is the case of asymmetrical mitoses in neoplasms. The

<sup>4</sup> If a chromosome is but a chromosome without character or distinction, there is in this case a violation of the rule stated. But if the nature of the element be significant, the rule holds, for the entire history of the supernumerary chromosome demonstrates that it is of a different order from the euchromosomes. It may in many ways depart from the history of typical chromosomes and finally end in complete elimination. Arguments, such as Della Valle's, require that such a structure be considered identical with all other bodies called chromosomes, without in any way regarding its individual history of ultimate extinction.



clear cut description by Wilson of the failure of certain chromosomes to enter into synapsis is not an explanatory fact but another 'subhypothesis.' Similarly, early separation of conjugants, delayed divisions of certain elements, the presence of supernumeraries are merely so many condemnatory 'subhypothesis.' The exact determination of the union of the characteristic and well marked accessory chromosome, with a tetrad to form a multiple is not a fact at all, not even a hypothesis, but only a 'subhypothesis.'

From this it is clear that Della Valle, and others like him, have set up in their minds the conception of an absolutely fixed and invariable number of chromosomes of constant and unchanging form as the hypothesis of chromosome individuality. This is a man of straw, fathered by no biologist of standing or character. His destruction in no way affects the existence of the real image of a constancy in chromosome organization consistent with our other knowledge of the living substance of which these structures are a part. Such misinterpretation merely condemns the judgment of the one who has no better discernment of the real problem. It seems incredible that a biologist should be capable of twisting the truth in such a way as to turn an investigator's meaning entirely around so as to make his results support a converse. Only when his basic conception of chromosome constitution is fully appreciated does an explanation of Della Valle's attitude appear. This is expressed by him in several places in some such terms as these: The chromosomes are temporary and variable aggregates forming in the prophase and disappearing in the telophase ('09). Between two mitoses the chromosomes are so completely lost as individuals that finally no trace of them can be found ('13). It is easy to see that, with such a view of chromosome organization, no constancy is possible and the quickest way to dispose of variable numbers is to consider them the result of chance.

But such a disposition of the case neglects certain objective facts which can not be disposed of by consignment to the class of 'subhypotheses.' It has long been known that the accessory chromosome, and other heterochromosomes, preserve their mor-



phological identity from one generation of cells to the other. All through the spermatogonial divisions each chromosome lies in its own vesicle and can be clearly recognized. Recently Wenrich ('16) has carefully traced certain differentiated chromosomes in *Phrynotettix* through much of the history of the maturing germ cells, without loss of their physical identity. Instead of appearing as "temporary and variable structures" they are just the opposite, being persistent and exact in organization to the highest degree. Not only are the chromosomes constant in number, but also, and more fundamentally, the chromomeres. Such conditions are probably unknown to Della Valle and are certainly unappreciated or he could not make such sweeping assertions regarding chromosome instability.

Perhaps one of the most striking and extensive instances of variation in chromosome numbers yet reported is the one described by Miss Holt ('17) for the intestinal cells of *Culex*. Here, in one individual, there may be a range from six to seventy-two. Without extending the analysis of the case beyond the simple determination of the numerical conditions, one would be justified in believing that no significance attaches to the mere number of the nuclear bodies. This would follow on the major premise that indefinite and fluctuating variations in numbers are direct evidence of lack of definiteness in organization. This might presumably be admitted for the somatic cells without entirely invalidating the theory of individuality so far as it applies to the germ cells, for it is possible that, among other evidences of differentiation in cells, correlative changes in the nature and integration of the chromatin substance might occur. Especially might this be the case in pupal *Culex* intestinal cells, because these are on their way to disintegration in preparation for the new epithelial lining of the enteron. Meanwhile the germ cells of the species preserve an unvarying constancy in number. But, as Miss Holt shows, the matter is not so simple as it appears. True, there is variation, but it is not indefinite and unordered. There are not always six chromosomes present, but, if more occur, the numbers are multiples of three and repeat in corresponding series the sizes of the primitive complex. Not

only this, but the grouping is maintained and the derivatives of each of the original series remains associated with its fellows and, with them, forms a common vesicle. So far as prophase and telophase conditions are involved it would not be observed that anything unusual in numerical relations obtains. Even in the matter of this multiplication the chromosomes exhibit individual differences, and one may have advanced in the process beyond the others and in this way produce a total number not strictly a multiple of three. But no matter how many or how few there may be, each one of the individual groups repeats the characters of the original member. How far removed from mere chance crystallization out of an indifferent matrix is the ordered and definite reproduction of distinct morphological entities which we see here. In the very face of what seems at first glance to be the grossest of variability there appears only another marked instance of exact organization. It is of interest to observe also that division of the chromosome may occur independently of mitosis, which suggests, in connection with the observation that the actual splitting of the chromosome is a prophase change, that mitosis may be more concerned with cell division than with chromosome separation.

### *3. Chromosome sizes*

If the total amount of chromatin is constant for each cell and if the number of chromosomes is unchanged, it might reasonably be expected that the same series of sizes would always appear. These conditions are certainly realized in the individual, where the closest correspondence exists between the series and between individually recognizable elements. That like correspondence exists between individuals seems probable when the seriation in the complex is noted. An exact determination of chromosome sizes is however a very difficult undertaking, owing to the variety of forms they assume. One is forced to estimate relative volumes, and an exact determination between two nearly equal sizes of different form can not be made with certainty. Fortunately the difficulty of comparing sizes is less in the case of the

larger elements, owing to their volume and more uniform shape, and we may be sure of the series in this part of the complex. Since it is between these chromosomes that combinations occur, the members may easily be identified. The value of the evidence with regard to the constancy of chromosome organization as shown by size relation is best appreciated by a comparison of the different classes shown in plate 2.

In the one containing twelve chromosomes (1) there is a gradual increase in size from the smallest to the largest without any marked breaks. Class 2 shows the same condition, with the exception of the multiple chromosome, but if the presence of the accessory chromosome there be disregarded, the disproportion vanishes. Class 3 however presents a different aspect. There is the same gradation up to the largest element, which, in turn, is more than twice the size of the next one of the series. Similar conditions prevail in each of the other classes, with additional distinctness in classes 4 and 5 where the element succeeding number 8 is like the largest in class 3. In each of these cases there is an abrupt and disproportional increase in size, if these structures are regarded as simple units, which entirely disappears if their multiple constitution is recognized. Such jumps of size in the complex are lacking in any species of this family where the twelve chromosomes are present, and it is not reasonable to consider that they represent any great change in the organization of the chromosome such as would be involved if these huge chromosomes were in fact of the same value structurally as the lower ones of the series. However much difficulty there may be, for example, in locating chromosome 4 or 5 in the series, there can be no mistake made in judging the relative size of the largest element in classes 3, 4, 5 and 6. It is also easy to see that either half of this ring exceeds the dimensions of the next chromosome in size in somewhat the same degree as chromosome 11 does chromosome 10 of class 6. Similarly the members of the smaller ring in class 5 are related to chromosome 8 of class 6. The relations in size of the upper members of the series are demonstrated most conclusively by a comparison of them in classes 4, 5 and 6.

While these unequivocal conditions certainly obtain and justify the effort to homologise the chromosomes on the basis of size, it must be admitted that there appears to be some fluctuation in volume, both absolute and relative, between the same members of the series in different individuals. How much this may signify it is difficult to say. There are so many ways in which the size of a chromosome may be affected in the processes of microscopical technique that a just estimate of the significance of size variation is hard to reach. Thus it is known, for instance, that very marked differences in size may result from the method by which the animal is killed. If this be true, there are doubtless other ways in which these delicate structures may be similarly affected. Since however the animals themselves may vary greatly in dimensions without in any other way modifying their character, the same may be true of their chromosomes.

A very careful study needs to be made of chromosome dimensions, based upon material prepared with the utmost refinement of technique and studied with most careful mensuration. Under ordinary conditions there are many circumstances which would serve to obscure differences between nearly related members of a series and make their identification uncertain. While this is true, most of our observations would indicate that agencies affecting the size of chromosomes operate more or less uniformly on the entire complex, so that a proportionate change is found in each element. With the subject as difficult as it is there is no wonder that views as widely divergent as those of Meek and Della Valle exist. The former author thought at one time that he had detected a uniformity so great that only two diameters appeared in all the Metazoa, but a more extended experience did not confirm this opinion. It is true that there is much uniformity in the diameter of the chromosomes of a complex, and Meek's careful measurements disclosed this; but, on the other hand, there are extensive movements in the chromatin substances and the diameter varies inversely with the length. There is always the possibility of such movements and the extent of them at any given time is variable, so that we may expect to find differences in dimension although the volume remains constant.



Meek has done good service in pointing out the facts, but it is unfortunate that he should push his generalizations so far upon a limited experience. Very different are the methods and conclusions of Della Valle.

Variations in size, according to Della Valle, fall within the limits of observational error and are of the order of size variations of droplets in an emulsion. Correspondence in size between chromosome and the nucleus finds its explanation in the physical condition of adsorption. As in the case of numbers, the appeal is from an explanation based on order of determinable character to one in which only the chance circumstances of physical relations obtain. Opposed to this conception stand all the careful investigations in which definite size relations are shown to be preserved, not only in a single series but in a duplicate one. Within this occur occasional inequalities, which, in turn, are of definite character, pertaining to certain chromosomes and so recurring as to be accounted for most readily through genetic continuity and chance distribution and recombination. Upon this point again the circumstances in *H. viridis* offer important evidence. The smaller members of the complex form an ascending series which, in class 1, is continued without breaks through the larger members. But in the remaining classes there are sharp breaks in seriation of exactly the same character in all the cells of the individual, entirely beyond the range of observational error and past the possibility of chance variation. The conditions are altogether of the character of order and system common to living structures and foreign to purely physical phenomena. The material upon which the Italian author worked is not nearly so favorable for size determinations as are many other kinds, because of the great length and irregular contour of the elements, but even here much more accurate results could have been obtained by the exercise of such care as was employed by Meek. From some studies being pursued in this laboratory by Mr. Parmenter I feel certain that the Amphibia are no less exact in their size relations than are other groups of animals.



*4. Chromosome forms*

In a previous paper ('14) I have considered at length the subject of chromosome forms in the Orthoptera and do not here desire to go into the subject beyond what is necessary to an understanding of the multiples. A glance at plate 2 will show how strongly these elements differ from the tetrads in metaphase. The typical Orthopteran chromosome is telomitic and, in polar views of the spermatogonium and second spermatocyte metaphase, it appears as an approximately straight rod which, in the anaphase, becomes divided into daughter rods of similar nature. In the first spermatocyte these rods, joined in pairs to form tetrads, show a great variety of shapes in metaphase, but in the anaphase again become reduced to the form of two rods joined at one end to form a simple V, the enclosed space of which lies between two chromatids of the former tetrad. Upon division of the hexad in the first spermatocyte of *Hesperotettix* this condition is again realized for the rod-portion, but opposite this in the other anaphase group stands a V, the arms of which are not simple rods but double ones, and the enclosed space of this lies, not between chromatids, but between whole chromosomes. The nature of this double-V I have considered fully in former papers. Its peculiarity of form is due to the fact that fiber attachment is non-terminal—a condition resulting from the union of non-homologous chromosomes and not from a real shift of the fiber. All this is very clear in *Hesperotettix*, and the character of the double V is beyond question and easy of determination. From this one would be inclined to generalize and to say that where fiber attachment is shifted from the terminal position, producing double V's in the first spermatocyte anaphase, it is due to the union of non-homologous chromosomes. Of course to make this a valid argument there would have to be a corresponding reduction in the group number of free elements. This condition is fully met in *Hesperotettix* and is confirmed by the differential morphological characters of the elements involved. In the absence of such criteria based upon structure, the sole test regarding the character of the double V's would be that of numerical conformity.

Upon turning to *Mermiria bivittata* we encounter these conditions: a spermatogonial complex of twenty-two free elements among which are two atelomitic V's. If it were assumed that the latter are always multiples, then the male diploid complex would be twenty-four. There are two arguments against this conclusion however. First there is the circumstance that the genus—a well marked one—has species with the normal twenty-three free chromosomes. The value of such evidence has already been considered. A second piece of evidence is the actual morphological structure of the V's. One of them shows by all its history that it is directly comparable to the similar structure in *Hesperotettix*—it is a multiple composed of the accessory chromosome and a rod-shaped euchromosome. The other V is found in the first spermatocyte attached to this multiple element. Unless this combination be of greater valence than a hexad, the second V can be only a dyad. Strong evidence that this V is indeed only a simple dyad appears from a study of certain individuals which I have called the 'green' *bivittata*. In these specimens the multiple chromosome is almost the shape of the one found in *Hesperotettix*, the fiber attaching near the free end instead of toward the middle (figs. 58, 59, 60, pl. 7). The valence of the part is very clearly indicated in this way. Owing to mistaken enumeration in the spermatogonium, because of insufficient material, I regarded the multiple as a decad instead of a hexad in my earlier work. In this opinion I believed myself confirmed by the persistent type of chromosomes in the family. It was only when new material revealed to Miss Carothers that, in a full complex of twenty-three chromosomes, there might be numerous double V's, and that one such an element might be united to a rod, producing a J-shaped tetrad, that I found it possible to account for the form of the *Mermiria* multiple. With plenty of favorable material I was also able to determine accurately the diploid number of chromosomes in the male to be twenty-two. Having all these facts it was not difficult to solve the problem.

It is apparent, therefore, that form alone is not sufficient to establish the nature of a chromosome. The double V may or

may not be a multiple. In *Mermiria*, in the same chromosome of the first spermatocyte, we have two such V's, one of which is multiple, the other not. In *Trimerotropis* double V's are numerous although the complex is not reduced in number. But the form of the chromosome, taken in connection with the number in the complex, the relative sizes of the elements, their structure and behavior, is important. With these limitations on the value of chromosome form in mind, I wish to consider the evidence they afford regarding the nature of the *Stenobothrus* type of rings and V's in *H. viridis*.

In such a study the occurrence of the incomplete ring is very helpful because it constitutes an intermediate form between the two rod condition and the ring. An examination of these elements in classes 4, 5 and 6 (plate 2) will demonstrate these relations. In the last class, chromosomes 9 and 10 are rods arranged on the spindle parallel with its axis, and there divide at the middle constrictions. Similar structural conditions prevail in class 4, with the exception that, in place of chromosomes 9 and 10 as free elements, their position in the size series is occupied by a single chromosome in the form of a V placed in the same relation to the spindle. Upon division, the elements of this unusual chromosome separate at their middle constrictions just as did free chromosomes 9 and 10, and in one of the anaphase groups exactly the same conditions obtain as in cells of class 6 (fig. 28, pl. 5). The other group has, in place of two rods, a double V corresponding in the number and size of its subdivisions to the sum of those in the two rods from which it separated. All the circumstances of the case indicate that the only unusual condition here is the union of chromosomes 9 and 10 at one end, producing, in the diploid condition, a V, whose arms are unequal in size and otherwise non-homologous. The origin of such a combination has not yet been determined, but it is not a temporary condition during the maturation period, for it is found in all generations of the cells and is undoubtedly present in the somatic cells. Since it is present in half of the sperm it may as readily perpetuate itself as the two rod condition. If like relations exist during the maturation of the egg, then a means

for producing all the chromosomal arrangements found in *H. viridis* is at hand. Thus a two rod spermatozoon and a two rod egg would produce a complex having chromosomes 9 and 10 free as in classes 1 and 6. A double-V spermatozoon and a two rod egg would bring about the condition shown in class 4 where these chromosomes are joined at one end, while such a spermatozoon, united with an egg of similar composition, would result in the complete ring of class 5. It seems evident therefore that the forms of chromosomes found in the male germ cells may be accounted for on the basis of persistent fusions, segregations and recombinations.

Similarly, if chromosomes 11 and 12 are compared, we find the two rod, incomplete ring, and ring, and the argument for their relation is the same as for the preceding case. There are however two notable differences between the series. Of the six classes, only two show multiples of chromosomes 9 and 10, while four classes have combinations between 11 and 12. Again, the incomplete ring is as frequent as the complete in classes 4 and 5, while only one individual out of twenty-six belonging to classes 3 to 6 failed to have a union of the two tetrads at both ends. Thus we learn that the tendency of the large elements to unite is more pronounced and complete. In this connection it may be noted that in class 2 the largest element, although failing to form a union with a euchromosome, is united to the accessory chromosome. Only four individuals of the thirty-seven show this element not joined to another chromosome. Of similar import is the combination between the accessory with the largest free euchromosome in class 4. It is possible that the largest free tetrad is involved in class 3, although I judged it to be next to the largest when the drawing was made. Another thing to be noted is that only tetrads of nearly equal size unite together. The accessory chromosome is never joined to a chromosome of lesser size.

The form of the multiples, as represented on the tabular series of plate 2, is the one best adapted to serve as a means of comparison between them and the free chromosomes of other individuals. This relatively simple configuration is, however, not



the only one assumed, and some of the figures would be difficult of conception in the absence of acquaintance with the possible range of chromosome movements. For a number of reasons these variable forms are of importance and require consideration. It is to be noted that this extended ring may be the final shape assumed by other metaphase forms just previous to the anaphase, or it may represent the status attained by chromosomal movements antecedent to the metaphase. Probably all that is involved throughout is a difference in relative movements of the chromatids. An examination of figures 14*b*, 16*c*, plate 3 will reveal a chromosome, one part of which is extended over the spindle as are the members of the flattened ring already described, while, embraced between its ends, lies another ring within the plane of the equatorial plate. By comparing this chromosome with others shown in photomicrographs Q-V, plate 8 it is seen that this second ring may be progressively reduced until it corresponds in form and position to the extended element to whose ends it is attached—the sole remnant of the equatorial ring being an enlargement at the middle of the curved rod. It is apparent that the enclosed spaces in these two rings, lying at right angles to each other, can not be the same morphologically, unless the chromatids in synapsis have shifted. One must represent an interchromosomal space, the other the longitudinal split of the chromosomes. In this respect the element is similar to the double ring figures in certain Orthopteran tetrads.

Another form of not infrequent occurrence is similar in outline to the hexad of *Mermiria* and consists of a curved rod applied conformably to the spindle, with the fibers attached at the ends. From these ends also there spring at various angles more or less straight chromatin rods equal in length, approximately; to half the curved rod to which they join. Superficially such an element is almost the counterpart of the *Mermiria* hexad, but is nothing more than the large extended ring whose shorter half has divided at the equatorial constriction in advance of the longer (fig. 24). A more puzzling case is that of the curved rod extending from one pole of the spindle to the other and almost equalling the combined length of the two halves of the ring or V (fig. 19, pl.



4 and photomicrographs P., Y., pl. 8). Such a structure would result if the V were unfolded on the spindle with fibers attaching at each end, but in this case the parts on either side of the equatorial constriction would be unequal and division at this place would mean separation of whole tetrads. Because of the relatively slight difference in size of the joined tetrads, I have not yet been able to satisfy myself of the exact relation of parts in this elongated chromosome, but I suspect that separation of chromatids has occurred in a plane at right angles to that which obtains in the V. A full study of the prophase history should clear up this matter.

Emphasis needs to be placed upon the statement already made that the mere form of the chromosome is not conclusive evidence, either of its valence or its method of division. Valence can be determined only by a study of the element through the various generations of cells and in numerous individuals of the species. Planes of division are certainly identified only by some morphological character which marks one of the synapsed elements. Instances of the exactness in configuration of chromosomes of tetrad, hexad and octad valence have already been given, but it would seem desirable to consider the ring chromosome a little more at length. In former papers I have called attention to the importance of such elements as indicators of the planes of division, and in one ('14) I pointed out the distinction between the Hippiscus and Stenobothrus types and indicated how failure to recognize such distinctions has led to much confusion. At the same time it was recognized that, in the presence of parasynapsis, the opening in the ring might represent either the space between homologous chromosomes or their longitudinal division. Since there was, so far, nothing to suggest two types of ring formation, it was considered that only one exists and the available evidence would indicate that the annular space lies between homologous chromosomes. In the presence of one type of ring there must necessarily be two forms of first spermatocyte division, for the Hippiscus ring divides along the length of the ring while the Stenobothrus type separates transversely. Owing to the fact that, up to that time, all rings of the Stenobothrus type

has been found in complexes of less than the full twenty-three numbers, some uncertainty existed regarding the connection between these two circumstances. That this connection was not necessarily causal was indicated by the case of the unidentified 'Stenobothrus-like' species with a complex of twenty-one and numerous rings of this species-type. But not until the work of Carothers on *Trimerotropis* was it certain that there is no necessary relation between the *Stenobothrus* ring and lesser chromosome numbers. It is now clear that this form of chromosome is not in itself evidence of multiple constitution above the tetrad. Structurally all that is involved, apparently, is an atelomitic fiber attachment.

But if it is not true that such rings and the double-V's which unite to form them, and which reappear upon their division, are criteria for octads, it is very suggestive of such composition to find them so frequently in cases of reduced numbers. Where it can be shown that progressive steps in such unions occur within a species, as in *H. viridis*, and that morphologically recognizable divisions of these elements which, if counted as units, exactly restore the reduced number to that characteristic of the species and family, then the form of the chromosome is of value in determining composition. Especially is this true if by such means always the exact number characteristic of the group is restored, and if, in groups of different chromosome numbers, the distinctive numerical relations are preserved. All of these desiderata are realized in *Hesperotettix* of the twenty-three chromosome family and in *Jamaicana* of the thirty-three and thirty-five group as reported by Woolsey. The parallel between the two cases seems to be very exact, with the exception of a more marked tendency toward combinations in *Hesperotettix*.

The circumstances appear unequivocal in the cases of *Hesperotettix* and *Jamaicana* where multiples may or may not exist within one species and where intermediate steps indicate the method of combination. The question which next presents itself is with regard to the application of these criteria to cases, such as *Chorthippus*, where there appears to be a permanent and fixed reduction of number, not only for the species, but also for the

genus and related genera. In a recent paper Robertson ('16) has considered this matter with great care and has advanced strong arguments in support of the position that the family number may be maintained in the face of apparent reduction. His argument may be thus summarized. (1) The number of chromosomes for the family is twenty-three in the male; (2) the form of the chromosomes, when uncombined, is that of a rod with terminal fiber attachment; (3) size relations are constant and valid indications of homologies; (4) in the presence of less than the typical number of chromosomes certain ones of the complex are V's with non-terminal fiber attachment; (5) if the limbs of the V's are counted as units, the number twenty-three is restored; (6) the behavior of the limbs of the V's in maturation is parallel to that of corresponding free elements in other species of the family; (7) the morphological composition of the V is indicated by a non-chromatic bridge at the angle where the fiber attaches.

I believe that in the species studied by Robertson the criteria are largely applicable and the conclusions essentially justified. The evidence is, to a considerable extent parallel to that of *H. viridis*, with the exception of intermediate steps in linkage.

There is clearly a permanent reduction in the number of free chromosomes—it then becomes a question as to whether there is a definite loss of morphological entities in the chromosome complex, or whether all the twenty-three elements are potentially present, even though in combinations. It must be recognized, I think, that, however fundamentally alike are the cases of *Hesperotettix* and *Chorthippus*, they stand on a somewhat different basis morphologically. In the former, independence of certain chromosomes is strongly evidenced by their free condition in some individuals and their partial union in others; in the latter the number of free elements is constant, not only for this genus but for others nearly related, and evidence of combinations must be more indirect. Considering the criteria which I have employed in *Hesperotettix* and *Mermiria* I would regard the following as probably applying in the case of *Chorthippus*: (1) The number of chromosomes, so strongly indicated in the

family, is made exactly normal by counting the limbs of V's as units, (2) the graded size series, sharply broken if the V's are considered as simple units, is restored if they are compound elements, (3) the form of the univalent chromosomes is so generally a rod in the family that the occurrence of V's, in connection with the circumstances in (1) and (2), is strongly suggestive of multiple constitution. To these should be added the conformatory evidence of cases like *H. viridis* which establish on direct observation the principle of chromosome combinations.

That these circumstances justify the generalizations which Robertson draws regarding the multiple nature of V-chromosomes in general seems much less certain. Because of the great importance which attaches to the exact determination of chromosomal conditions in the germ cells it is essential that our cytological evidence be most carefully considered. I should like therefore briefly to indicate where it appears that limits should be placed upon the extension of the principles indicated by conditions in *Hesperotettix*, *Mermiria*, *Anabrus* and *Jamaicana*. Since Robertson has made the widest application of these by his conception of the multiple constitution of V-shaped chromosomes, an examination of his presentation will illustrate the nature of the difficulties attending such generalizations, into some of which I was myself led. It is fortunate in this connection that there is no question regarding the accuracy of the observations. His work is very carefully done and the drawings are clear and definite.

Agreement has already been indicated with the first two premises of his argument, i.e., that the number twenty-three is typical for the family, and that the form of the univalent chromosome is a rod with terminal fiber attachment. Like most other biological generalizations, however, these are not without exceptions in the *Acrididae*. *Pamphagus*, with telomitic elements, has only nineteen chromosomes and *Trimerotropis* with numerous and variable V's has a full complex of twenty-three. My unidentified *Acridian* reported in a former paper ('14) (which may be a species of *Circotettix*) has nine atelomitic chromosomes in twenty-one chromosomes, while *Circotettix*, as



reported by Miss Carothers, has similar conditions of less than the typical family number and more than enough V's to compensate. Despite these exceptions the value of the evidence for constancy of number and form is strong because of the few departures from type.

With regard to size as a test for homology there is much less certainty. No chromosome is more definitely to be determined than the accessory chromosome and this falls in no constant size position, either for the family, genus or species. Robertson reports it varying in size from number 1 to number 5 in the Tetigininae and from number 8 to number 10 in the subfamily Oedipodinae. It seems to vary from number 3 to number 5 in the genus *Hesperotettix* and from number 4 to number 5 in the species *viridis*. Variation in size of the individual chromosomes, recognizable by other morphological characters, has been reported by Wenrich ('16) after a most careful study of *Phrynotettix*. In this latter case variation appears to be the result of actual loss of certain parts of the chromosome, but in general it is due to the extent of condensation and possibly to the amount of more fluid substance held within the chromosome. All our observations indicate the existence of a fairly definite series of sizes throughout the group, and the extreme members of the complex can not be confused, but it is clear that no certain identification of neighboring elements can be made by size alone. For this reason, even if the achromatic bridges did mark the limits of joined chromosomes, it would not be certain that specific numbers united as is indicated by Robertson. Here it may be noted that in *H. viridis* chromosomes of nearly equal size, and the largest in the series, are joined together. Practically the same conditions are reported by Woolsey in different species of *Jamaicana*. Judging from her figures I should be inclined to say that it is not improbable that neighboring chromosomes, and those of the largest size, are united here. Measurement of the chromosomes as drawn is not a very accurate way of determining their size, since the amount of foreshortening can not be known, but it is so frequently the case that the element marked '14' in her figures of the multiple, is clearly larger than the free number '15,' that the possibility of confusion exists.



The significance of the form of V-chromosomes, as an indication of multiple constitution, is bound up intimately with the question of numbers. Only when the limbs of the V, counted as units, with the rod chromosomes, total the number characteristic of the family can there be such evidence of multiple constitution, according to the terms of Robertson's argument. If it appears that such elements exist under other circumstances, then it is necessary to assume that there may be more than one type of V. This Robertson does and uses as a criterion for the V, indicative of multiple composition, the presence of a distinct achromatic bridge at the apex of an acute angled structure. The final test of the validity of his generalization becomes therefore a structural one. There is no doubt of the accuracy of his determination of such a condition and he has with great care traced it through all the stages of maturation. It is unfortunately true, however, as Carothers and Wenrich have shown, that equally clear non-staining bridges occur in atelomitic chromosomes of *Trimerotropis* where the full complex of twenty-three is present. Conversely, in cases where undoubted combinations exist, as in *H. viridis*, no distinct achromatic bridges at the point of fiber attachment appear. This seems to be true of *Jamaicana* also, judging by the figures of Woolsey and of Robertson himself. It seems certain therefore that the V-form of chromosomes, is, in itself, no indication of a multiple condition. If this be true for the *Acrididae* it is quite useless to consider its value in other groups where the chromosome constitution is less well known.

The form of certain first spermatocyte chromosomes and the behavior of their parts in this mitosis is regarded by Robertson as further confirmation of his belief in their multiple character. He draws a close parallel between them and free elements of other species through homologies of size and form. Since the limits of these supposed elements are fixed by the achromatic bridges at the point of fiber attachment, their validity as tests for chromosome boundaries is questioned for the same reasons as were given above. I should however like to speak more at length regarding this form of chromosome—the double rings.

These are represented very exactly in Robertson's ('16) figures 174, 179, 180 and 182, and the achromatic break is considered the point of union between tetrads. I believe that the weight of evidence supports this interpretation: that these are indeed potentially multiple chromosomes, but at the same time I feel that there is no justification for the belief that this form of chromosome is the result of the union of two tetrads of such shapes that this configuration results. My evidence for this is that just such shapes occur in full complexes of twenty-three chromosomes, of both telomitic and atelomitic type. They are found in many species and have been figured by Sutton, Granata, and myself. As I have pointed out, the two rings lie in planes perpendicular to each other and their origin is easily conceived if the chromosome is constituted of four parallel rods. A similar conception appears in the interpretations of the other two authors cited. The same difference between double rings of the telomitic and atelomitic types holds as in the case of simpler annular chromosomes of the two types, for the fiber attachment is persistent and determines the position of the chromosome on the spindle. Therefore such structures are very much alike in *Trimerotropis* and *Stenobothrus* and are so placed on the first spermatocyte spindle that the ring to which the fiber attaches lies parallel with its axis, while in forms with telomitic chromosomes only, such as *Hippiscus*, *Tropidolophus*, *Brachystola* and others, the ring to whose 'lugs' the fibers attach lies in the equatorial plate. Such differences are however entirely independent of simple or multiple composition of the diploid chromosomes.

Finally I consider them valueless as criteria of multiple constitution, because in *Chloealtis*, a genus nearly related to *Chorthippus*, the same numerical and size relations obtain, and there are no double rings to suggest preservation of chromosome forms such as are found in more widely removed genera. So far as the form of the chromosomes is concerned, the conditions in the *Tryxaline*, *Chloealtis*, are much more nearly like those in *H. viridis*, of the *Acridiinae*, than they are in *Chorthippus*, a very nearly related genus.

At this point it may not be out of place to speak of the condition of cross segmentation as an index of chromosome boundaries, since this is a matter that has led many, including myself, into mistakes. These non-chromatic segments are doubtless definite indications of structure in the chromosome and probably are always of the nature of the spaces lying between the chromomeres, as in the 'selected' chromosomes described by Wenrich ('16). Such regions are more pronounced in appearance and more persistently free of chromatin where the fiber attaches than elsewhere in the chromosomes, but probably do not differ otherwise. Even this distinction may be lacking, as in *Mermiria* (figs. 58, 59, pl. 7) where the chromosomes seem not infrequently to separate at different levels. It was this strong cross segmentation at definite levels, with subsequent division, that led me to believe in the decad constitution of the multiple in *Mermiria*.

Another thing which has contributed to Robertson's misinterpretation of the V-chromosome is his belief that only in their presence do pachytene loops appear in the nucleus. No one feature is more characteristic of the prophase condition in all *Acerididae* (from which the *Tettiginae* should be removed, as I suggested in 1908 and as Robertson believes) than the bending of the chromosome so that both ends lie at one side of the nucleus. Even the univalent accessory chromosome takes this position, as was shown in an earlier paper ('02, fig. 12). Wenrich finds that the looped condition is not confined to the large elements, but is characteristic of the small chromosomes of which he made careful, detailed study. It is of interest to note that, in this respect, the members of the octad in *H. viridis* behave as though they were independent, each forming its own loop. This is a very strong indication, indeed, of their multiple nature. The conditions at this time in the *Tettiginae* are unique so far as my experience goes. Here, as Robertson states, the chromosomes are extended in a more or less straight line, and since they are much longer than the nuclear diameter, the whole nucleus is drawn out, producing very irregular shaped structures. It

was this circumstance, of which he had detailed knowledge, that led Robertson to believe that loops are unusual.

The form of the chromosome is conceived by Della Valle to be uniformly that of a rod with rounded ends. Such is frequently the shape of metaphase chromosomes, particularly in the diploid condition, but no one who has studied the complicated changes through which the members of the haploid series pass at the synaptic period, could entertain the simple conception of a homogeneous rod spontaneously dividing along its length, advocated by this author. The most remarkable and striking phenomena of biology are entirely ignored by this explanation. Precision of organization, shown by Wenrich to extend to the limit of our observational powers, is entirely disregarded by the gross conception of a formed and homogeneous rod cleaving like an unorganized colloid mass. The extensive and significant changes of the prophase, when the division actually occurs, receive no recognition in this crude explanation of chromosome division. The form of the chromosome is a much more involved question than would appear from the statement of the case by Della Valle.

#### *5. Chromosome behavior*

Of very great importance for the theory of chromosome individuality is the behavior of the elements united into multiples. If the chromosomes are merely colloid masses—products of the cell—it would not be expected that they should exhibit any differential characters. The very fact that such differences of behavior exist is a disproof of all theories which postulate complete loss of identity and reformation of chromosomes at each mitosis. Unless there is continuity of substance there can not be continuity of organization, because we know of no organization independent of material basis. Besides, such persistence of structure and substance is observable. As indicating the high degree of this specificity of chromosome characters, the multiples are of much value, especially when they involve the accessory chromosome. If all the elements of a mitosis are

'identical' and 'homogeneous,' as Della Valle asserts, there would not be the marked difference in behavior of the euchromosome and the accessory chromosome which we always witness in the Orthoptera. Most especially would this be true where the two types constitute a single mitotic element. What a contrast the actual facts regarding exactness and differential character of the chromosomes present to the crude and vague generalizations of those who conceive the cell to be only a microscopic laboratory for the play of the chemical forces involved in colloidal crystallizations. It is however fortunate that there are minds not content with a half way denial of the facts relating to nuclear constitution and the substitution of indefinite generalization about the activity of the cell as a whole or of specific enzymes, etc., but which push the denial of precision of organization to an unconscious *reductio ad absurdum* in some field of chemistry. For through this service we are brought to see the contrast between clearly defined facts, gained through researches of the most exacting and painstaking character and the uncertain and contradictory theories springing from impatient and prejudiced minds.

The facts regarding the differential behavior of the chromosomes, together with those relating to numbers, sizes and forms, are, many of them, now commonplaces of observation and are regularly determined by beginning students in microscopic anatomy. Others, such as those exhibited by the euchromosome multiples described in this paper, are no less striking and significant. They all speak clearly and unequivocally in favor of an order, in terms of morphology, and against one of a more remote and molecular character. But no single feature of chromosome behavior possesses the significance and fundamental importance which attaches to the unquestioned reproduction of each individual chromosome during mitosis. Why, unless there be something in the organization to be preserved, should the elements so carefully reproduce themselves down to the most minute structural peculiarities? It is inconceivable that the complexity and exactness of this process should exist unless there is some correspondingly important function to be carried out.



If the chromosomes are homogeneous and identical, as Della Valle asserts, their substance could most easily be divided while they were in 'solution.' The complication of the mitotic process is all out of proportion to the simple end to be served under such an assumption.

#### 6. *Chromosome distribution*

In most mitoses it would seem that every step of the process is directed toward securing the exact distribution of the chromatin elements. The outstanding exception to this rule has been the independent movement of the accessory chromosome in one of the maturation mitoses, where it goes into one cell entire without division. This is clearly a differential mitosis, featured by an asymmetrical distribution of the chromosomes. One of the elements possesses a distinctive character not shared by the others—it has an individual and more or less independent movement which takes place at only one time in all the history of the organism. Up to this one point it is distributed in mitosis like the other chromosomes, but just here it betrays the inherent difference of its nature. So much of the chromatin substance acting as a unit in mitosis, possesses distinctive characters. Are these the consequence of this separate unity, or is there some specific nature of the material? The history of the hexad multiple chromosome answers this question definitely in favor of the latter alternative, for, although joined to another element, the same characteristic features of behavior and distribution mark the accessory chromosome as when it is free. It is incomprehensible that there should be this definiteness of action on the basis of mere chance—such a conclusion is foreign to all our experience with living structures. Taken in connection with the parallelism between the development of sex and sex linked characters, and its distribution during maturation, all the history of the accessory chromosome speaks for specific organization and self perpetuation. The fact that it violates the practically universal rule of a complete division of all the members of the complex in each mitosis in order to accomplish its necessary distribution and self perpetuation is an unanswerable argument

for its specific individuality. That this distribution is properly maintained, although the element is no longer free in the multiples, is only another confirmation of the conclusions otherwise determined.

Another evidence of specific organization through acts of distribution comes from the studies, upon unequal and heteromorphic chromosomes by Miss Carothers ('17). Organization, expressed in size or form, is perpetuated by a distribution and a conceivable recombination according to chance movements. There is no indication that any indefinite variation exists. While it is true that no explanation of the origin of the heteromorphic condition is strongly suggested by the facts so far observed, it may be noted that Wenrich's work affords objective evidence regarding inequalities in size. Always a means for the maintenance of given conditions appears in the mechanism of mitotic distribution.

The same precision in distribution which marks the accessory chromosome is true also of the elements which unite to form the euchromosome multiples. No difference exists between the distribution of these members of the complex, whether free or combined. It might be thought that the intimate and apparently permanent association of two chromosomes, resulting in the formation of a new mitotic unit of different value, would materially change the activities of the components. So far as I can see this is not the case, and even the details of movements in the prophase are preserved in the face of the multiple condition.

The problem of accounting for the occurrence of a given variable series of heteromorphic chromosome combinations in Trimerotropis has been considered by Miss Carothers and she properly arrives at the conclusion that segregation and chance recombination of components offer a competent explanation of the observed phenomena. At the same time there is not excluded the possibility of a readjustment of relations between the chromosome complex and the new cytosome at the time of fertilization, which, within a limited range of possibilities, would result in a similar variable series. The conditions within the

species of *H. viridis* would not be fully met by the explanation through segregation and recombination, without assuming a heterogeneous character of the material or a lack of survival of certain classes. While both of these possibilities exist they are remote, and the phenomena, taken in their entirety, are much more suggestive of a reconstitution of relations in the new individual, probably at its origin on fertilization. This is a problem which concerns the most fundamental activities of the chromatin substances, and about which we lack information on all the stages of fertilization and maturation of the egg. Breeding experiments should serve to decide between the possibilities suggested, and these will be carried out, but the nature of the processes will not be clear until the complete cycle of the chromosome complex has been studied. This fact has been realized since the beginning of the work on the Orthoptera, but the technical difficulties involved are great and so far have not been overcome.

While, therefore, it is not possible to draw any definite conclusions from our present knowledge, it may be desirable to direct attention to the conditions which confront us. Reasons are given elsewhere for considering the individuality of the chromosomes as well established by the observed uniformity of number, size, form, behavior, etc., but in the case of the multiple chromosomes we face a series of variations. As has been pointed out, variability is a universal property of living matter and is a subject for investigation, not an evidence of lost individuality. Taking into consideration, first, the accessory chromosome, whose individuality is marked so definitely as to be unquestionable, we find, that, within the species *H. viridis*, it may possibly vary slightly in size although this may be only apparent. From this we would draw the conclusion that, for the species, size is a fairly safe criterion of homology, although not absolutely exact. Using size to identify homologues, then, we discover that the accessory chromosome is joined to the largest chromosome in class 2 (plate II), to the third or fourth from the largest in class 3, and to the fifth from the largest in class 4. With persistent union, segregation and chance com-

bination it is apparent that there could be no union between a spermatozoon of class 2 and an egg of classes 3 or 4, for instance, without synapsis of non-homologous chromosomes. Still using size as a test for homology, we find no such indication of non-homologous chromosome unions in this species.

This difficulty entirely disappears, however, if we assume that the formation of multiples takes place at fertilization. Such a view is in strict accord with our knowledge of the importance of this step in the production of differences between individuals and of constancy of character exhibited by each of these variants throughout its subsequent history. Also there is offered a ready explanation of the formation of multiples, which would, at the same time, explain the variety of elements sometimes involved, as in this case; for if there be a period at which chromosomes are brought into intimate relations in a linear series, multiples would be merely persistent cases of such associations. The contrary might also be true, the multiples showing a tendency to overcome forces keeping the chromosomes apart, but, in view of the variety of elements involved in *H. viridis*, this would seem less reasonable than the first explanation. There are also other considerations, into which I have not the time to enter here, which incline me to believe that multiples indicate the operation of a common principle of chromosome association. The alternative for this explanation of the conditions in *H. viridis*, by reason of associations established at the time of the union of the spermatozoon and egg complexes, is the assumption that, at some time, each of the classes represented in plate 2 became established in some way and has since maintained itself by the segregation of its elements and their chance recombinations, with the elimination of all the unrepresented conditions. By this we are still left without an explanation of the first instance of each class and must confront a selective mortality of considerable magnitude, for which there is no evidence.

Such an explanation, as that to which I incline, for the conditions of the multiple chromosome in *H. viridis* does not make impossible another for the case of *Trimerotropis*, and it may



well be that in the latter instance, which concerns questions of fiber insertion or other variation between homologous chromosomes, the simple method of segregation and chance recombination obtains, while in *H. viridis*, where non-homologous chromosomes are involved, another mechanism is operative. Genetical experiments will shed some light on these difficulties and it is hoped that these may be completed before long.

### *7. Chromosome individuality*

As has been stated elsewhere in this paper, the circumstances relating to the multiple chromosomes are strong proofs of the theory of chromosome individuality, as indeed are all facts indicating order and definiteness in form and behavior. So far as I can see there is no half way ground between the assumption that the chromosomes are definite, self-perpetuating organic structures and the other which presents them as mere incidental products of cellular action. According to one view individual chromosomes are descendants of like elements and possess certain qualities and behavior because of their material descent, the visible mechanism for which is the process of mitosis: according to the other any similarities that may exist in the complexes are the result of chance aggregations of non-specific materials. It is a choice between organization and non-organization in the last analysis, at least in terms of cellular structures. To attempt the substitution of a conception of molecular organization, which is beyond the experience of the biologist and which exceeds the present powers of the chemist to analyse, is to cast aside all hope of solving the problem of cellular action, because it is necessary to understand, not only the physical and chemical phenomena involved, but also their different forms in the various parts of the cell. It is readily admitted that these are not the same in the nucleus and in the cytosome, but some hesitate to recognize differences between parts of the nucleus, and more are disinclined to grant specific activities to the chromosomes.

Since it is not possible to observe directly the action of the chromosome we are obliged to make use of indirect evidence,



seeking parallels between elements of structure and action in the chromosomes, and the mass effect of cellular action as exhibited in the so-called body characters. Such a method is justified by all our other experience in tracing relations between structure and function in organisms, and while it apparently resolves the individual into parts of greater or less independence, has given us our best conceptions of it as a whole. Homologies have been much more significant of relationship than analogies when concerned with organic parts, and I do not doubt that conclusions drawn from structural relations will greatly exceed in value the remote and equivocal analogies between nuclear structure and colloidal phenomena. On the basis of our experience we anticipate a commensurate relation between structure and function, but this does not mean a fixed and exclusive correspondence—it does not signify that a function necessarily entirely lapses in the absence of a certain structure. All our observations indicate that the relatively few functions of the living substance—irritability, contractility, metabolism and reproduction—are shared by all its parts in varying degrees, and when we speak of the function of any somatic organ or cell part we mean its outstanding and preponderant activity.

With this understanding of the relation between morphology and physiology, we speak of the chromosomes as having to do with the process of reproduction, and conceive of them as non-homogeneous within themselves and individually unlike. In this sense they bear factors, or have parts, which are most concerned with certain peculiarities which, through cumulative action in cell reproduction, come to fullest expression in regions of the complete soma. Through what manner a factor operates to produce a cell structure which, in a given cell aggregate, summates in the form a somatic character, we do not know, and that is not the question immediately at issue in the individuality hypothesis. What is postulated there is that the chromosomes are self-perpetuating entities with individual peculiarities of form and function to identify them. Characteristics of form and behavior we see; certain very definite parallels between these and the manifestations of somatic characters exist

beyond question; provision for the perpetuation of the organic unity of the individual chromosomes is found in the process of mitosis; the actual direct result of its operation appears in the uniform conditions of the complex in the individual animal; the extension of this beyond the organism to the group and the means for it in the phenomena of maturation and fertilization are easily established by observation; the age old existence of all these circumstances is revealed by the near approach to uniformity in the chromosome complex of the multitude of species of unnumbered individuals constituting a family. And yet, in the face of this overwhelming mass of evidence indicative of order, system and specific chromosome organization, some conceive only the action of ordinary chemical forces, or the chance association of indifferent substances, while others, over impressed with the thought of a general coordinating force in the organism, deny significance to the orderly play of its cellular parts.

While opponents of the individuality hypothesis differ thus widely in what they would substitute for it, they almost invariably seize upon one supposed condition of the chromosome as a basis for discounting the remaining positive evidence which is presented for the hypothesis. Even some well informed cytologists who accept the implications of the facts, regard this circumstance as a severe weakness in the chain of evidence. That the chromosomes do not maintain a compact and easily recognizable form in the interval between mitoses is accepted by many such biologists as proof that they no longer exist as entities. All the other manifold indications of character and continuity do not weigh against this apparent loss of identity. Doubtless it would be more satisfying if we could at all times perceive the chromosomes in unchanging form in all stages of cellular activity, but why we should demand this condition as a test for individuality in the chromosomes when we unhesitatingly admit the unity of an organism in all the varied changes of its development from a single cell, through such complexities of change and metamorphosis as to give rise to doubts of even the phyletic position of some stages, it is difficult to see. Being organic, the chromosomes must change their form, they must

suffer division of their substance and they are obliged to restore this loss through metabolic changes. Since these changes of substance take place at surface contacts there is an obvious advantage in increased superficies and, in common with other, larger structural elements, the chromosomes become extended and their substances are diffused. In this state their boundaries may not be well defined and this circumstance has been seized upon as a disproof of their continuity.

For myself, even were the visible limits of the chromosome completely lost, this would not appear as a convincing disproof of persistent individuality in the face of the large number of facts pointing in the opposite direction. Of such facts those relating to the history of the multiple chromosomes, outlined in this paper, are of great importance. Their bearing upon the various aspects of numbers, sizes, forms and behavior, has been mentioned under these different headings. At this point however I wish to summarize this evidence upon the general topic of individuality. But before doing so I should like to repeat statements previously made that the accessory chromosome in the germ cells of the male presents us with the history of a particular chromosome, at all times distinct and well delimited, whose physical identity suffers no eclipse during the various metabolic changes of growth and division. It is readily distinguished where it occurs, not alone in a given cell, but in all the germ cells of the species, genus, family and order. The same element, always recognizable by reason of its structural character, is identified in different phyla and is shown to have a definite relation to the development of sex characters. It would seem impossible to conceive any more definite marks of individuality than is possessed by this chromosome, in whose history there is no confusion of character or break of continuity, and yet it does not receive even mention in some attacks upon the theory of individuality.

Again, its position may be granted, but with the reservation that it is unique and no criterion for other chromosomes. Upon this point it is now merely necessary to say that the accessory chromosome differs only in degree from the euchro-

mosomes, and general conclusions regarding the nature of chromosome organization may properly be drawn from its history. That its condition is indeed not unique is shown by various approaches to it on the part of certain chromosomes in *Phrynotettix*, as demonstrated by Wenrich ('16). The great similarity to the euchromosomes is also indicated by the union with the accessory chromosome in multiples, and by its entirely typical nature in the female when paired.

The mere academic question of individuality is not here important, the practical matter before us is to decide whether the metaphase chromosomes of two cells are individually identical organic members of a series because they were produced by the observed reproduction of a similar series of the parent cell, or whether the resemblance is independent of this genetic relation and due to chance association of indifferent materials, or to a reconstituting action of the cell as a whole. It is my belief that the observed act of reproduction, by which the organization of the chromosomes is materially transmitted in each mitosis, together with all facts indicating extensive distribution of given conditions, definiteness of organization, uniformity of behavior and consistency of deviation from the normal, are so many clear indications of the individual character of the chromosomes. Transmutation of form, even to an extreme degree, can not be held as a valid argument against a persistent individuality. A consideration of the criteria applied to larger organic aggregates well supports this view. Such objects are said to possess individuality when they exhibit a more or less definite unity which is persistent and characterized by peculiarities of form and function. Most clearly defined is this individuality when it may be perpetuated through some form of reproduction to find expression in new units of similar character. The term does not connote unchangeability, and there may be fusions with more or less loss of physical delimitations, followed by separation, even after exchange of substances. The test of individuality is material continuity, but it does not necessarily involve complete or entirely persistent contiguity. An organism may bud off new individuals similar to itself, the substance of its body



differs from time to time, movements of parts take place, fragmentation occurs, extreme attenuation or extension of substance is found, even separation and recombination of parts may happen and yet the individual maintains itself. What it may have been in the past, what its possibilities of future development are, what potentialities of multiplied individuality it suppresses do not affect the reality of its individuality. It is, as Huxley says, "a single thing of a given kind." If one such thing divides into two, there are two individuals; if two unite into one indistinguishably there is a single individual; if a fusion of two things occurs in part, without loss of physical configuration, there are still two individuals in existence. Only when the substance of one thing disappears or becomes incorporated integrally into the organization of another does its individuality depart.

If all these variations of physical state may occur in the history of an organism without sacrifice of individuality, there can be no reason for urging them against a conception of the individuality of the self perpetuating chromosomes. Especially is this true in face of the facts recorded here and in other papers showing the high degree of chromosome constancy, for any given period, in all the attributes by which we usually judge individuality. The conditions in *Hesperotetix* and *Mermiria* at first seem to be a contradiction to this generalization, but, when examined with care, show, not an instance of fundamental change, but merely of modified detail. From these results it is very clear that a chromosome number less than normal for the group is not a necessary evidence of lost elements. In a similar manner the studies of Miss Holt on the intestinal cells of *Culex* indicate definitely that numbers in excess of the normal do not signify the addition of anything not previously represented. Loss or gain in chromosome numbers is a condition to be investigated, not implicit evidence of an altered organization with loss of organic individualities. That the chromosomes are not the ultimate structural units is, however, indicated in many ways, but it remained for Wenrich to show the nature of this more minute portion of the chromosome architecture. From



his results it would appear that all chromosomes are, in a sense, multiples, and it is conceivable that these parts might be variously associated, producing even extensive numerical variation, still without loss or gain of essential structural elements.

The peculiar case of *Ascaris megalocephala*, where evident multiple chromosomes are retained intact in the germ cells while they suffer fragmentation in the somatic cells, is an extreme instance of numerical variation which occurs in one individual. How common such relations may be we do not know, since so little has been done in the study of the complete history of a single organism; but it is obvious that the Orthoptera stand at the opposite extreme from *Ascaris* in the persistence of chromosome organization, for here all the cells of an individual seem to be invariably the same, and the individual is representative of the group. But even in this stable assemblage we encounter the conditions which I have described for *H. viridis*. This case, with its diminution in number, and that of *Culex*, with its great increase, are alike in the fact that the measures of difference are entire chromosomes and not parts of such, as seems to be the case in *Ascaris*, unless indeed these chromosomes be multiples of a high order of complexity.

If it were possible for chromosomes to reproduce themselves and still preserve their physical configuration unchanged, there would probably be little question of their continuity and individuality—the demonstration would be self evident. But it happens that the necessities of the case require that each newly produced chromosome should take part in the formation of a new nucleus, through whose activities the cell as a whole and each chromosome, individually, is enabled to restore the volume diminished by the act of division. During this process the outlines of the chromosomes become materially changed and in their extreme diffusion can no longer be traced in many cases. Because of our limitations in observational power they appear to be lost as separate individuals and we are thus deprived of the simple test of observed continuity. Later, in the same cell, there reappears a series of chromosomes severally like those which seemed to disappear during the period of metabolic activity.

We confront two alternative explanations for this re-integration of the chromosomes; either they actually persist as discrete units of extremely variable form, or they are entirely lost as individual entities and are reconstituted by some extrinsic agency. There is no other possible explanation and we must weigh the evidence for one or the other of the alternatives.

All facts which indicate order and system in chromosome features speak for the former, those which demonstrate variability and indefiniteness, for the latter. The case for discontinuity is strongest in the absence of any chromosome order, and becomes progressively weaker with the establishment of definiteness and precision in form and behavior. Evidence has already been given in other papers on the Orthoptera to show the exactness in organization of chromosomes, their persistent continuity between mitoses in certain cases, the uniformity in number and behavior through large groups, and the parallel behavior of chromosomes on one hand, and of somatic characters on the other. In this paper are discussed what appear to be the widest departures from normal conditions so far found in the group. Accepting as most important, next to actual observed continuity, the evidence afforded by constancy in number, size, form and behavior of the chromosome, I have been able to show that these criteria are here applicable and that variation is only apparent and not real. It seems to me that the facts disclosed in *Hesperotettix* and *Mermiria* not only fail to weaken the case against individuality, as tested by constancy in number, size, etc., of the chromosomes, but, on the contrary, greatly strengthen it, for the reason that all of the distinguishing features of the individual chromosomes are maintained, even in the presence of unions into multiples of higher order. The fact that, although the number of independent units is reduced, nothing is lost from the complex, is most important and significant.

Finally, the main feature of chromosome individuality must not be lost in a discussion of whether it is the chromatin or the linin that persists with less change. However great in importance this distinction may be, it does not alter the aspect of the problem of individuality in its present phase. One or both of

these substances may be responsible for the integration of the chromosomes, but it is the fact that there is a definite structure to be identified which concerns us most. The substances of the chromosome divide into new units of similar relative sizes, and these structures, now of half the original content, take up unlike materials and replace the missing portions. The complexes of the daughter cells are now equivalent to that of the mother unit. It is obvious that, since the chromosomes, as such, divide, all of their substances must be involved. There may be some question whether one may be lost as a morphological entity while another persists—although this is much open to question—but something maintains the organization which we recognize in the chromosome and it is this organization which we study. In view of the fact that our microchemical tests are so far from specific in their action, it is not the part of wisdom to build very extensive theories upon their evidence. It is quite possible that one substance, in different phases of its activity, may present alternately the aspects of chromatin and achromatin, and some conditions of the staining reaction suggest this, so that it would be unwise to involve the whole question of chromosome organization in a dispute regarding the nature of their substance, based merely upon uncertain staining reactions.

#### *8. Chromosome specificity*

That chromosomes might be genetically continuous and still be of the same nature, without differential character, is, of course, conceivable. In fact it is more than probable that, in a sense, they are all alike, for they probably share the general properties or functions of protoplasm. But that they carry out these activities in the same manner is a conclusion quite foreign to the evidence. Just what the nature of this differential action may be has not yet been discovered, but the action of the sex determining chromosome and the groupings of characters in *Drosophila* are suggestive. Male and female possess the same series of parts, and the difference between them is one of relative development. Nothing unique for either sex exists and such a thing

as an 'exclusively male' or exclusively female character is not to be found. The evidence here would indicate that the 'factors' for maleness, are such controls of the developmental processes as will eventuate in a certain degree of differentiation of each cell of the body. The sum of all these, in any somatic region, produces a condition which we call a male character, and the total complex of these constitutes an individual which is a male. In another individual the same series of elements obtains, but each cell and each part is slightly different and the sum total of the characters produces an assemblage which we recognize as a female. Should the conditions be varied, even by the internal secretion of the gonad, in some cases, these may be altered to resemblance of the opposite sex.

However much the entire complex is involved in the production of characters which are called sexual, it is apparent from the history of the accessory chromosome in the Orthoptera that it is a differential agent. All other things apparently being equal, the presence of one accessory chromosome so shapes the developmental processes that a male results, while if two are involved a female is produced. In some way, not now apparent, the action of this particular one of the chromosomes differs from all the others in producing an effect of which they are not capable—that is, it has a specific action. Apparently it is unlike them in being concerned with the entire body, including the germ cells, which are differentiated into eggs or spermatozoa. A further indication of the specific nature of this particular chromosome is afforded by sex-linked characters whose development is conditioned by exactly the same circumstances of distribution as those which mark the alternatives of maleness or femaleness. The facts relating to the accessory chromosome are the strongest evidence we have in support of chromosome specificity, because the history of this element is so clear, its continuity so unbroken and its relations to certain characters so definite. But any evidence for specificity of one chromosome is, at the same time, support for the general conception of the differential nature of chromosomes, and for this reason the facts concerning the accessory chromosome have additional value.



The only direct evidence for any such a differential nature of the other chromosomes is afforded by the work on *Drosophila*. In this case a whole group of characters follows the rule of distribution governing sex. This is the only element that can certainly be identified, but the groups of observed characters, not sex linked, correspond to the three remaining pairs of chromosomes, and the numbers of characters in each group are in proportion to the size of the chromosomes. Of these one pair is very small and there is a corresponding small group of characters. As is well known, the analysis of the conditions in *Drosophila* has gone so far that the relative loci for the different factors have been calculated. It would seem from these results that there is every reason to regard the chromosomes of this fly as qualitatively different. While there is no correlation of this sort known for the Orthoptera, the actual history of the chromosomes which their germ cells exhibit affords a mechanism adequate for the facts of alternative inheritance and for the segregation and chance recombinations of characters. While the multiple chromosomes do not afford any direct evidence for specificity of function, it would seem, because of all the facts, that the purpose for which these studies on the Orthoptera were begun—a correlation between germ cell structure and somatic characters—is much more feasible of accomplishment than appeared at the beginning.

#### V. SUMMARY OF RESULTS

1. Chromosomes are definitely organized chromatic bodies acting as units in mitosis.
2. These units are of unlike morphological value in the different generations of germ cells.
3. While any one cell generation is marked by one general type of chromosome organization, individual chromosomes may differ from the type in higher or lower degree, by definite steps or intervals.
4. In a given species the integration of the chromosome complex may vary from individual to individual.
5. For any one individual this integration is fixed.



6. Despite this individual variation in the composition of the mitotic units, there is no loss in the total morphological elements of the specific complex, or departure from the usual habits of synapsis and segregation of homologous elements.

7. The maintenance of the original complex in the presence of a variable number of mitotic units results from associations of these to produce others of higher valence.

8. Where variation between individuals exists for any one cell generation, it is the result of differences in association between certain definite mitotic units.

9. Such variations are not indefinite, but occur in a fixed order between certain units.

10. The occurrence of associations between units of lower order into those of a higher results from the operation of a common integration principle.

11. The union of homologous chromosomes to produce tetrads is temporary and is terminated in one of the following maturation divisions; multiples of non-homologous chromosomes are permanent for the individual and the union does not end in either maturation mitosis.

12. Associations in the first spermatocyte of a higher order than tetrads are the result of lower associations which are persistent throughout all cells of the individual.

13. Associations into tetrads in the first spermatocyte occur between homologous chromosomes.

14. Higher associations than tetrads in the first spermatocyte are necessarily non-homologous.

15. Associations may be complete and persistent, or only approximations.

16. Associations may be complete in parts of chromosomes and incomplete in other regions.

17. The point of union between associated units in metaphase is at the ends of the chromosomes.

18. In telomitic chromosomes this is at the point of fiber attachment.

19. Atelomitic chromosomes of unit value have not yet been observed in multiples, except in that part of the tetrad of *Mer-miria* not joined to the accessory chromosomes.

20. Multiple chromosomes are of two types (a) those between euchromosomes and heterochromosomes and (b) those between euchromosomes alone.

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## DESCRIPTION OF PLATES

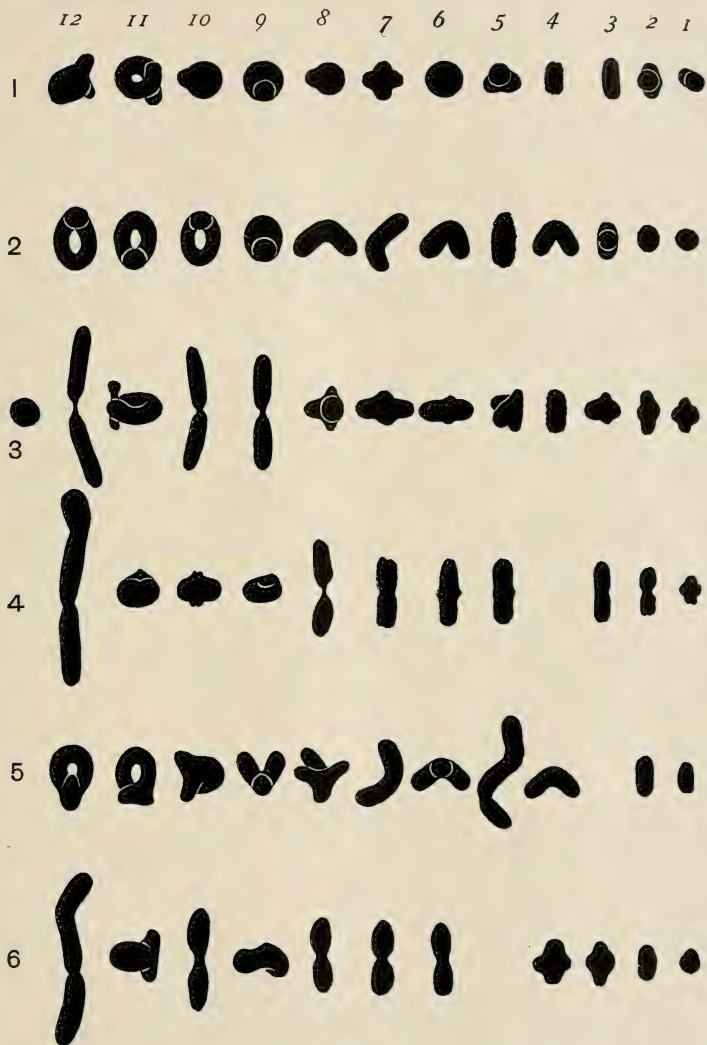
All figures are drawn at an initial magnification of 2860 diameters under the camera lucida, and appear in the reproduction at a magnification of 1800. The photomicrographs were obtained with a Zeiss 2 mm. objective of 1.40 N. A., projection ocular 4 and a Watson 'Holoscopic' oil immersion condenser. The original magnification of 1000 diameters is here reduced to 666, except fig. IX.

## PLATE 1

## EXPLANATION OF FIGURES

Horizontal rows represent complete first spermatocyte complexes, usually in lateral view, numbered 1 to 6. These are arranged so that chromosomes of homologous size are brought into vertical rows, numbered from 1 to 12. Blank spaces in vertical rows represent the normal position of the accessory chromosome which is joined to a tetrad.

- 1 A complex of *Hesperotettix brevipennis*. All twelve chromosomes are free.
- 2 *Hesperotettix festivus*, polar view.
- 3 *Hesperotettix viridis*, class 1—twelve chromosomes plus a supernumerary.
- 4 *H. viridis*, class 2.
- 5 *Hesperotettix pratensis*.
- 6 *Hesperotettix speciosus*.

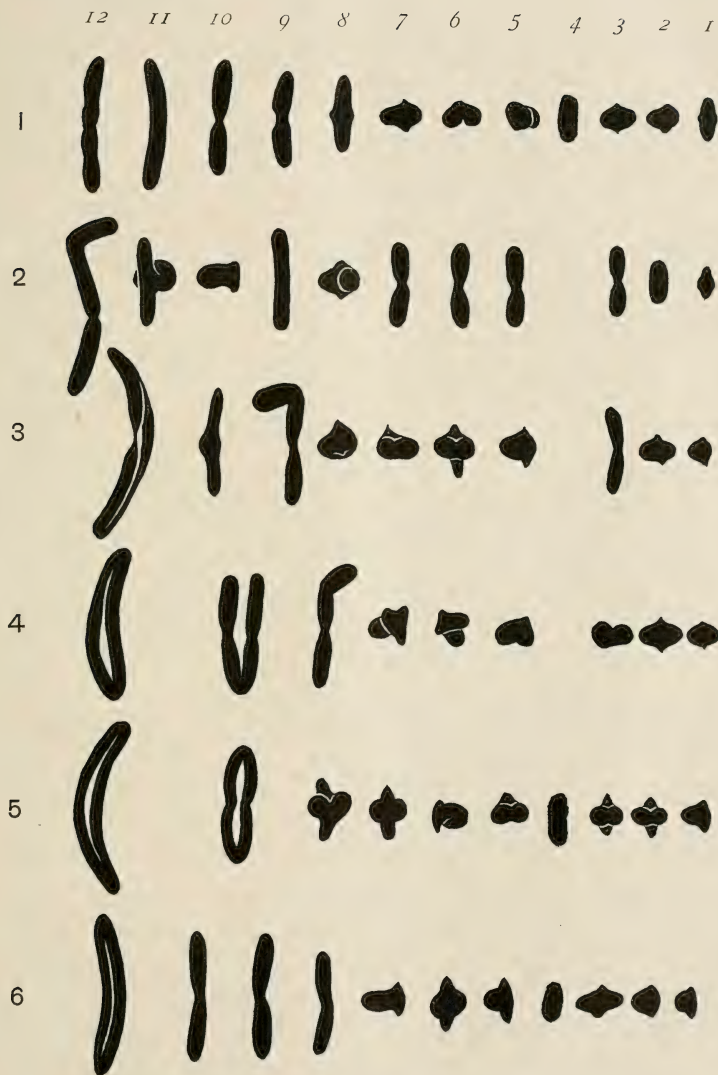


## PLATE 2

### EXPLANATION OF FIGURES

Complexes, arranged as in plate 1, of representatives of the various classes of first spermatocytes in *Hesperotettix viridis*. The classes are indicated by the figures at the left, 1 to 6. Euchromosome multiples are arranged between the rows to which their tetrads belong. Not all conditions are represented here but may be found in other figures. Thus the smaller octad of class 4 may also be a ring in some individuals; the smaller ring of class 5 may be a V; and the ring octad in one individual of class 6 is a V.





## PLATE 3

### EXPLANATION OF FIGURES

1 to 5, 7 to 9 Polar views of spermatogonial metaphase complexes. 1 to 7 are of *Hesperotettix viridis*, 8 of *H. brevipennis* and 9 of *H. speciosus*.

1, is of class 2, showing twenty-two chromosomes, of which one is a multiple. The accessory chromosome portion of this is marked 'X.'

2 An example of class 3. The members of the large first spermatocyte euchromosome multiple ring are the larger V-shaped elements, while the smaller V includes the accessory portion of the hexad. Twenty separate chromosomes.

3 A complex of class 4, in which the largest two V's are parts of the first spermatocyte ring, the next largest one of the octad V, and the smallest of the hexad. Nineteen separate chromosomes.

4 In this group, representing class 5, there are three V's belonging to two octads of the first spermatocyte, the smaller of which is a V. The accessory chromosome is free. Twenty separate chromosomes.

5 From an individual of class 6 with a free accessory chromosome and one octad ring in the first spermatocyte. Twenty-one chromosomes.

6 An anaphase group of class 4 showing the 4 V's (fig. 3 above).

7 A complex of class 6 in which the octad of the first spermatocyte is a V instead of a ring. The one V in this spermatogonial group is involved in the formation of this multiple. Twenty-two free chromosomes.

8 From *H. brevipennis* in which all specimens studied showed twenty-three rod shaped chromosomes.

9 The one V in this complex of twenty-two chromosomes will constitute a part of the hexad in the first spermatocyte. The accessory chromosome forms one limb.

10 An octad ring of *H. viridis* in the late prophase. It will commence to separate at the apex.

11 Late prophase condition of such a chromosome as shown in figure 10.

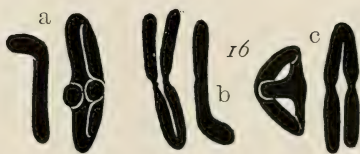
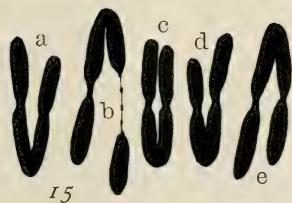
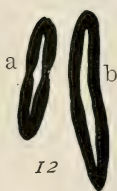
12 Shows metaphase condition of such octad rings. These are from different cells.

13 Two metaphase octad rings of *H. viridis* from the same cell. Class 5.

14 Similar elements from another cell of the same individual as shown in figure 13. The large octad *b* has one tetrad forming a ring lying in the equatorial plate at right angles to the other member. The small octad *a* shown edgewise.

15 Five examples of the V-shaped octad of class 4. The inequality of the constituent tetrads shows clearly.

16 Multiples of class 4; *a* the large octad and the hexad; *c* the two octads, the larger like figure 14 *b*.



## PLATE 4

### EXPLANATION OF FIGURES

17 Examples of octad rings in late prophase showing various aspects during the process of opening out. These are arranged somewhat in the order of progress from *a* to *k*.

18 Lateral view of first spermatocyte metaphase, class 6. One octad ring, and accessory chromosome at one pole.

19 Three examples of extended V's, such as shown in Photo Y.

20 Lateral view of first spermatocyte metaphase of class 5 with the V portion of the smaller octad going to same pole as the accessory chromosome. Ten free chromosomes.

21 A complex of a first spermatocyte of class 6 in which the one octad is a V.

22 Two first spermatocyte octads from one cell.

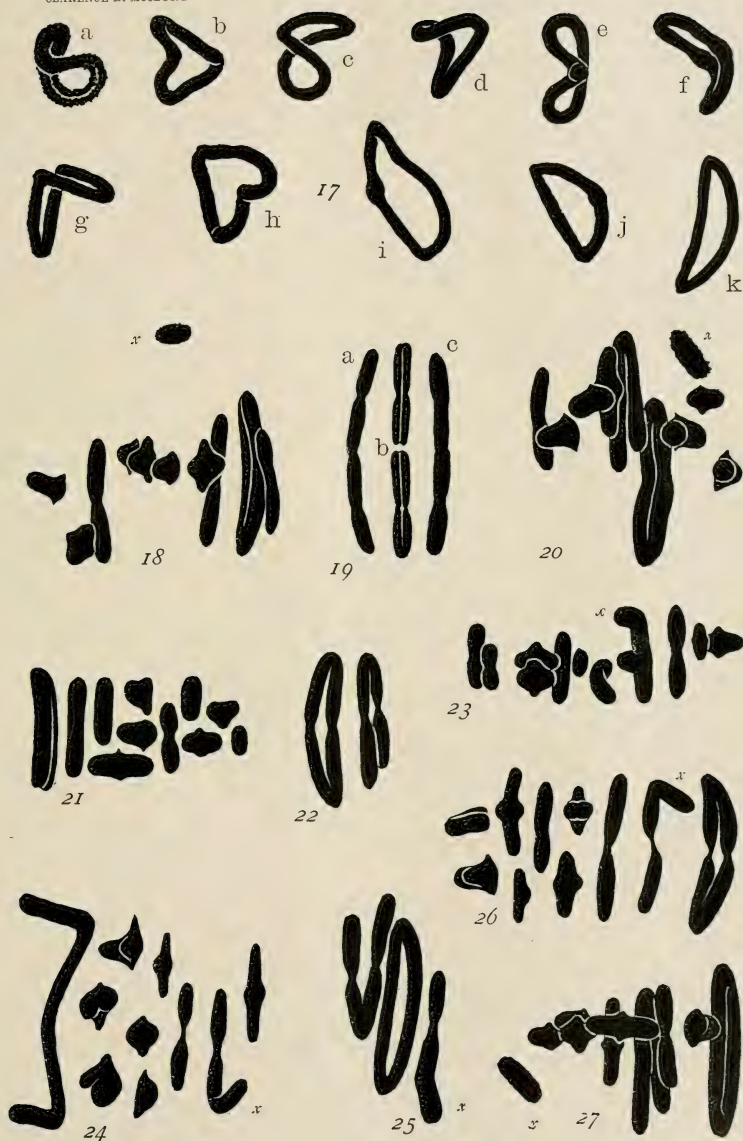
23 A complex of *H. pratensis* in which the hexad is similar in shape to that of *Anabrus*.

24 A complete complex of *Hesperotettix viridis*, class 3, in which the octad has opened at one side, simulating the appearance of the hexad in *Mermiria*.

25 The three multiples from a complex of class 4.

26 An entire complex of class 3, from the same animal as in figure 24.

27 A representative complex of class 5 with one V.





## PLATE 5

### EXPLANATION OF FIGURES

28 First spermatocyte anaphase, class 4 with one V, showing the distribution of the rod and V parts, in black. The entire complex, including the other multiples is shown.

29 to 42 Various second spermatocyte metaphase complexes (except fig. 33) in polar view, of *H. viridis*.

29 Class 1 with accessory chromosome.

30 Class 2 without accessory chromosome.

31 Class 2 with accessory chromosome in multiple.

32 Class 4, (one ring, one V and hexad in first spermatocyte) no accessory chromosome, two rods of V, and half of ring.

33 First spermatocyte anaphase of class 4, no accessory chromosome.

34 Class 6 (in first spermatocyte one ring, free accessory chromosome) no accessory chromosome.

35 Class 6 with accessory chromosome.

36 Same condition as figure 35, same animal.

37 Same condition as figure 35, another animal.

38 to 42 From one animal of class 6 with the octad in the form of a V, accessory chromosome free.

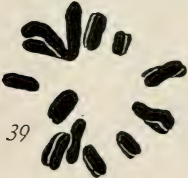
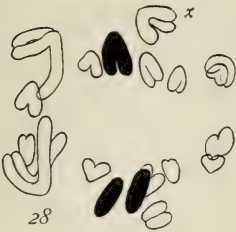
38 With V and no accessory chromosome—ten chromosomes.

39 With V and with accessory chromosome—eleven chromosomes.

40 No V and no accessory chromosome—eleven chromosomes.

41 As in figure 40.

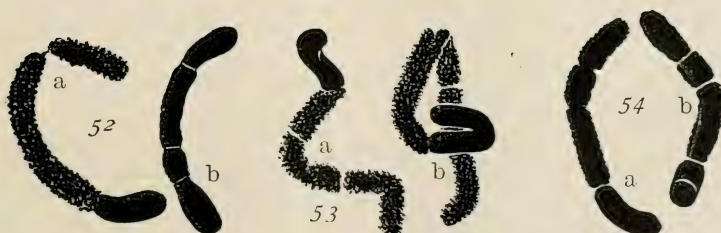
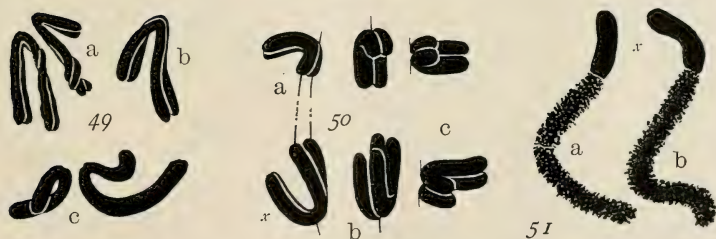
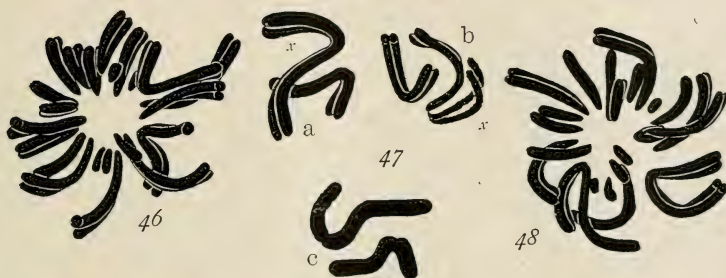
42 No V, but with accessory chromosome—twelve chromosomes.



## PLATE 6

### EXPLANATION OF FIGURES

- 43 to 54 From *Mermiria bivittata*.
- 43 Somatic complex of the female with twenty-two free chromosomes and two similar multiples, each containing an accessory chromosome. The euchromosomes are all telomitic.
- 44 A spermatogonial complex of twenty-two free chromosomes with two unlike V's, in one of which the accessory chromosome betrays its presence by its characteristic granular condition. The other V is smaller and is confined to the male line by always going into the opposite cell from the accessory chromosome in its segregation division.
- 45 Another female somatic complex.
- 46 Spermatogonial complex.
- 47 Pairs of spermatogonial V's in different stages showing disproportion in size.
- 48 A female somatic complex—one V foreshortened.
- 49 Spermatogonium, V-chromosomes. *a* and *c* associated pairs.
- 50 *a* The multiple in first spermatocyte anaphase, lateral view; *b*, *en face*; *c*, as these elements would appear in second spermatocyte metaphase.
- 51 Multiples in late first spermatocyte prophase showing the marked difference in appearance between the smooth, homogeneous, condensed accessory chromosome and the yet granular tetrad to which it is joined.
- 52 First spermatocyte multiples, *b* almost fully condensed and showing non-chromatic clefts. These are about in the relative positions of the fiber attachments and the plane of division.
- 53 As in figure 51.
- 54 Like 52 *b*.

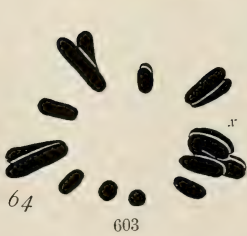
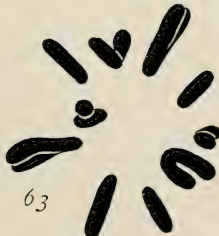
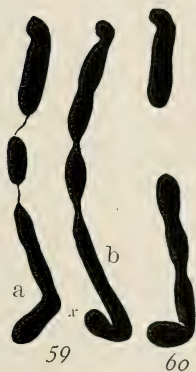
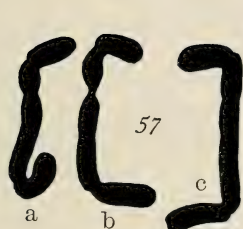
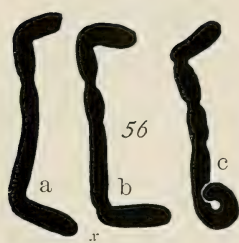
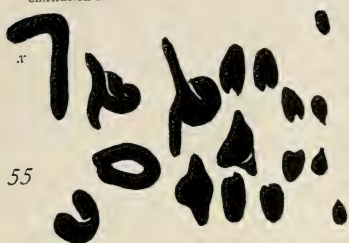


## PLATE 7

### EXPLANATION OF FIGURES

- 55 to 65 From *Mermiria bivittata*.
- 55 First spermatocyte metaphase showing the multiple and the five smaller chromosomes divided.
- 56 Three multiples of first spermatocyte metaphase.
- 57 Like figure 56.
- 58 Three multiples of the 'green' variety of *M. bivittata* in which fiber attachment is almost at the end of the bent portion. At *a* complete separation, in *b* and *c* beginning stages.
- 59 Similar to figure 58. Note the various constrictions, simulating a decad constitution, emphasized at *a* by separation at two levels.
- 60 Complete separation of bent rod opposite accessory chromosome.
- 61 Polar view of second spermatocyte metaphase.
- 62 Lateral view of same stage.
- 63 to 65 Polar view of second spermatocyte metaphase. All have eleven chromosomes, one of which is a V, but in one case, figure 63, this is a simple chromosome and in the other, figure 64, it is a multiple involving the accessory chromosome.





## PLATE 8

### EXPLANATION OF FIGURES

A to Z and II to III are photo micrographs of *Hesperotettix viridis* cells and IV to IX of *Mermiria bivittata*. A to D and IV to VI smears, the remainder sections.

A, B, C show the appearance of multiple rings with the longitudinal split and numerous clefts, D is a lateral view of a first spermatocyte metaphase showing the multiple ring in profile and the accessory chromosome at one pole.

E Polar views of spermatogonial metaphase with telomitic and atelomitic chromosomes.

F, G, H Profile views of hexad multiple.

I to N Lateral views of V-shaped multiples showing characteristics of form and movement.

O Ring and V. multiples.

P, A V-multiple with one limb out of position.

Q to T Ring multiples in which one tetrad has the plane of its ring perpendicular to the other.

U, V Late stages in the movement of such rings as appear in Q to T.

W, X Late metaphase stage of ring multiples.

Y An extended V similar to that of P.

Z A ring multiple about to divide.

II Anaphase condition of a V-multiple (cf. fig. 28).

III Late anaphase condition of a divided ring. The resulting V's are double.

IV Prophase condition of a hexad showing the accessory chromosome condensed and the tetrad thin and granular.

V, VI Metaphase hexads showing constrictions.

VII, VIII Profile views of hexads.

IX Lateral views of mitoses  $\times 400$ .





## MULTIPLE COMPLEXES IN THE ALIMENTARY TRACT OF CULEX PIPIENS

CAROLINE M. HOLT

*Simmons College, Boston, Massachusetts*

THIRTY-THREE FIGURES (FOUR PLATES)

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### INTRODUCTION

The phenomena discussed in this paper first came to our attention in the course of a systematic study of the chromosomes of *Culex pipiens* under the direction of Dr. McClung in the Zoological Laboratory of the University of Pennsylvania. The work was undertaken by a group of graduate students for the purpose of verifying certain accounts of this species already published. But in the course of the investigation so many interesting questions arose that it was decided to make a more thorough study of the cells of *Culex* than had originally been planned. Accordingly, after a general investigation of all the tissues, each student directed his attention to some particular phase of the work. At the same time, each contributed whatever came under his observation which would be of use to the others.

I wish here to acknowledge my indebtedness, first of all, to Dr. McClung for the encouragement, the invaluable counsel



and the thoughtful criticism which he has given so generously throughout the progress of the investigation; to Miss Eleanor Carothers to whom I am indebted for a part of the slides; and to my co-laborers, Dr. Phineas Whiting, and Mr. Robert Hance, whose reports on other phases of the study have appeared separately.

#### TECHNIQUE

Larvae and pupae of *Culex pipiens* were taken during the fall and winter from tubs of water in the University greenhouses and from the alligator-tank in the vivarium. These were kept in jars in the laboratory and a certain number of adults allowed to emerge, to be identified as a check in the identification of the pupae and larvae. Nothing but *Culex pipiens* was ever found in this material.

The observations for this portion of the work were all made upon sections of pupae. The abdomens were cut off just posterior to the thorax and immediately placed in fixing fluid. Some twenty methods of preparation were tried out, including fixation in six different modifications of Bouin and Flemming; with heat, cold, and room temperature; and for varying lengths of time. The method which gave the best results and which was finally adopted was as follows:

*Fixation.* One to six days at about 100° F. in "P. F. A. 3."

Picric acid 75 parts

Formalin 15 parts

Acetic acid 10 parts

Length of fixation after the first twenty-four hours seemed to make little difference in the results.

*Dehydration.* Very gradual, by drop method.

*Clearing* in xylol.

*Embedding* in paraffin.

*Staining.* Iron haematoxylin, twelve to twenty-four hours. Cleared in xylol and mounted in balsam.

All of the slides upon which this study is based are in the collection of the Zoology Laboratory of the University of Pennsylvania, except one prepared by Dr. Stevens and now in the Bryn

Mawr Laboratory. This material has proved favorable for the study of all somatic tissues as well as of the germ cells. It includes sections of a large number of individuals in which various parts of the alimentary tract are undergoing metamorphosis.

#### PREVIOUS OBSERVATIONS ON THE ALIMENTARY TRACT OF CULEX

M. T. Thompson has described in detail the changes which take place during the metamorphosis of the digestive tract of the mosquito. But while he notes the fact that mitotic figures are very numerous toward the end of these changes, he does not discuss the complexes.

Miss Monica Taylor, while seeing the great importance of working out the somatic complexes thoroughly, seems to have been handicapped by a difficulty in finding dividing somatic cells. She makes no mention of any variation in the number of chromosomes in the gut tract, and her figures show only cells previous to the beginning of metamorphosis or after its completion.

In fact, in all the work which has been done upon the chromosomes of *Culex*, observers have overlooked, or at least have failed to mention, an interesting phenomenon which occurs in connection with metamorphosis of the gut tract—the appearance of multiple complexes in cells which are about to give place to the new adult tissue.

#### BEHAVIOR OF THE GUT CELLS DURING METAMORPHOSIS

During the pupa stage, the entire gut, posterior to the oesophagus, undergoes metamorphosis. The larval tract degenerates and is absorbed, while the new tract is formed from cells, the nuclei of which appear between the bases of the larval cells. As the new epithelium forms, a marked activity develops in the old cells. There is some cell division accompanied by rapid multiplication in the number of chromosomes. While the normal somatic number in material prepared by the ordinary methods, is apparently three, as Miss Taylor ('14) states, the constant occurrence of splits in these chromosomes gives weight

to Dr. Stevens' view that we have here three bivalent chromosomes—the same condition found by Metz ('14) in *Drosophila*, where parasynaptic union of homologous chromosomes takes place between mitoses. Hance ('17) has demonstrated that the normal somatic number may, by improved technique, be clearly shown to be six.

The four normal complexes shown in figure 21, a, b, c, and d, may be interpreted in two ways. 21 d seems to represent an early metaphase before the final equatorial arrangement and division of bivalent chromosomes, while a, b, and c may be groups in which homologous bivalent chromosomes are separating and are about to pass to the poles; in fact in a, they are already moving away from the center. Under such an interpretation, the six chromosomes in a, b, and c, are in each case, bivalent, representing fused homologous elements. The enlarged ends, which not infrequently are split, suggest such a condition. The other interpretation of these four groups is that each of the six chromosomes in a, b, and c is a univalent chromosome, lying in the equatorial plate, ready for division. The work of other investigators indicates that the latter is the true interpretation. As in normal somatic cells, homologous chromosomes tend to fuse during the resting stage; so in the metamorphosing gut cells, the same condition apparently obtains.

In *Culex*, the degenerating intestinal cells frequently show thirty-six or forty-eight elements; and in one case, at least sixty-eight chromosomes appear in a metaphase group. This particular cell probably contains seventy-two, although it is not possible to determine the exact number (fig. 23, *y* and *z*). But whatever the number, approximately four divisions of half of each of the original chromosomes must here be represented, and three of the other half.

It is possible that cytokinesis does not always follow karyokinesis, though there may be an increase in the amount of cytoplasm, since some of the cells containing multiple complexes are larger than the normal (fig. 8). But, if this be the case, we might expect to find multinucleated cells, and there is no evidence of such a condition, save in a few possible instances where

cells are on the point of disintegration and cell boundaries can not be definitely determined. On the other hand, a cell containing a complex of three or four times the normal number of chromosomes may be of about the same size as one with six chromosomes (figs. 27 to 30). It would seem therefore, as if the growth of chromatin might be greatly accelerated in the 'resting stage,' followed by rapid splitting in the prophases, so that the chromosomes retain their normal size, while the cytoplasm grows, with or without division, at the normal rate. However that may be, it is evident that there is a great increase in the amount of chromatin in the old cells of the alimentary tract, preparatory to their absorption by the new.

#### MITOSIS IN MULTIPLE-COMPLEX CELLS

In the late anaphase of cells in which the number of chromosomes is not large, the chromosomes appear to lie in groups of three. Where the number is great, it is not possible to make out the exact arrangement, but from conditions in the early prophases, it is believed that the chromosomes pass into the resting stage in three groups, each group made up of the individuals derived from one of the three pairs of chromosomes of the original complex. Now and then a pair of chromosomes lags behind on the equatorial plate. An interesting case of this appears on Dr. Stevens' slide, on which such a pair occurs in several cells of the intestinal epithelium of the same individual (figs. 5 and 31). In telophase, the nuclear wall can not be distinguished, but the region of the nucleus is suggested by a light space about the telophase masses.

As the chromosomes pass into the 'resting stage' they gradually lose their staining power and become indistinguishable within an irregular mass taking a pale blue-gray stain with haematoxylin (figs. 3, 4, and 5). For a time, scattered bits of chromatin remain visible within this mass, but these eventually disappear, and only a pale nucleolus can be seen. The chromatin next makes its appearance in granules about the periphery of the nucleus and in faintly staining threads centering

in the nucleolus. At this period the nucleolus may take a pale stain so that the chromatin can be clearly seen collected about it (fig. 6) or, as the chromatin threads become more distinct, a deeply staining mass may appear in the region of the nucleolus.

The chromatin threads are gathered into an irregular mass which becomes three-lobed and finally resolves itself into three vesicles in which the granular chromatin threads are fairly distinct (figs. 7, 8, and 9). The vesicular appearance is then lost, but the chromosomes remain in three distinct groups of very long, folded, and somewhat twisted threads (figs. 10, 11, 12). These threads immediately begin to straighten and condense (figs. 11 to 16). This process of condensation is very interesting. Not enough cells at exactly the same stage of condensation could be found to make it possible to decide definitely, but comparison of the longest pairs of chromosomes in figures 13, 15, 16 and figure 14, and of the medium chromosomes in figures 13, 15, and 16 and of the short chromosomes (especially *c*) in figure 13 with the short one in figure 15, certainly do suggest very strongly that condensation takes place in a very definite and orderly manner, and that in all nuclei at the same stage of condensation, we should find the same number of granules in corresponding chromosomes. During this period the chromatin threads split. Whether, in the resting stage, the multiples of a given chromosome really become fused into a single thread, it is not possible to say. But the fact that in individuals which show complexes of thirty-six or forty-eight chromosomes in metaphase, early pro-phases in adjoining cells show but three or six splitting threads, with far too much chromatin for a like number of chromosomes, suggests that a parasynaptic union of sister chromosomes takes place in the telophase and also that the re-separation may come at any time during prophase (figs. 10 to 16 and 19).

There is nowhere any evidence of anything but a longitudinal splitting of the threads. Figures 10 and 11 appear to be cases in which an early division has occurred, while figures 13 to 16 show division taking place in threads in which the chromatin has become condensed to comparatively few granules. Since each normal complex is made up of three pairs of chromosomes, and



since the homologous members of each pair show a tendency to fuse and form a single bivalent chromosome, we should expect to find the chromosomes of the multiple complexes also conjugating, not in three pairs, but in three groups of homologous individuals. It is believed that such is really the case.

We find the fully condensed chromosomes still arranged in groups of three, the difference in size of the three kinds making it easy to see that we have sister chromosomes in each group. Compare figures 21 A, B, C and D for relative size of the three chromosomes in normal complexes. But the individuals of a group do not always divide at the same time, which accounts for the finding of nine, eighteen, and thirty-six chromosomes in some cells, whereas, simultaneous divisions of all the members of a complex would give twelve, twenty-four, etc. It is interesting to note that when an early division takes place in one of the members of a given group, the same thing occurs in the corresponding member of each of the other two groups. A good many complexes have been found in which this point is brought out very clearly through inequality in thickness of the individual threads. In figure 17, A and B are evidently sister chromosomes, possibly derived from the mate of E; C and D show the same relation to F. Figure 19 brings this out even better. A, B, and C evidently represent one pair of which one member has divided into two, A and B. Figure 20 illustrates the same point.

It is obvious, since every group counted belongs either to a 'six series' or a 'nine,' i.e., six, twelve, twenty-four, forty-eight, or nine, eighteen, thirty-six, seventy-two, etc., that, when irregularity in the time of division of members within a group does occur, it must come with the first splitting of half of the threads, and that otherwise all the derivatives of each of the daughter threads of the first split must divide simultaneously. If this were not the case, we should get multiples of numbers other than six and nine. We might suppose an early division of either the maternal or paternal element of each pair to have given us the nine chromosomes which we find in some complexes. The fact that in some individuals we find only the 'nine series'—nine, eighteen, thirty-six, could be explained by supposing the

maternal or the paternal group to be more precocious, either dividing earlier or, in the first division dividing twice.

The chromosomes grow so rapidly that in most cases the individuals of a multiple complex show the same sizes as those of 'normal' chromosomes found in the larval gut or the newly formed cells of the adult intestine (fig. 21 A). But even in metaphase we may find evidence of precocious splitting, giving abnormally slender chromosomes (fig. 23).

In the metaphase, we find the triplex arrangement still holds though here it is much more difficult to make out (figs. 24, 26, 27). In figure 26, for example, the two groups of six chromosomes on the lower side of the figure suggest that they may have been derived from the division of the small chromosomes of the original group. The individual at the extreme right seems a little larger and possibly belongs to the group above. The remaining twenty-four fall quite naturally into two groups. And so, with careful study, it has been found possible, in nearly all cases, to separate even the metaphase complexes into three component groups.

As in 'normal' cells, the chromosomes of the multiple complexes arrange themselves horizontally upon the spindle, where they split lengthwise and pass to either pole as V's (figs. 31 and 32. Compare with 'normal' somatic groups—fig. 21). Note even in multiple complexes the tendency on the part of the chromosomes to arrange themselves in triangular groups in metaphase.

#### DISINTEGRATION OF THE CELLS

After several multiplications of the chromosomes, the cells begin to show signs of degeneration. Apparently this usually comes after the third or fourth division. Vacuoles appear at first as lighter spots in the stained cytoplasm (fig. 8). Later these vacuoles have the appearance of empty spaces in the cell body, which is rapidly losing its staining power. The chromatin, meanwhile, becomes liquid and takes the form of black-staining drops of varying sizes, which may finally run together into one or two large masses. The nuclear wall breaks down (fig. 8)

and the chromatin flows out. In the lumen of the newly formed gut appear great numbers of vacuolated 'ghost cells' whose outlines can barely be discerned. And in the midst of these, streams of a gray-blue substance appear, filled with tiny black granules. This is evidently the chromatin from the disintegrating cells. All this material is apparently digested, for it has never been found in the very posterior part of the alimentary tract.

Such then appears to be the history of the cells of the larval gut tract while that tract undergoes metamorphosis. For a time, metabolism is greatly increased; disintegration follows, with final absorption by the newly-formed imaginal cells.

### *Discussion*

These facts with regard to the intestinal cells of *Culex* may possibly have some bearing upon two of the unsettled cytological questions at present under discussion—the 'Individuality Hypothesis' and the 'Kern-plasma Relation.' It might be urged that this is degenerating tissue and that therefore abnormal conditions prevail, but the uniformity and consistency in the behavior of all these intestinal cells lead to the belief that these conditions are normal, that they have a definite place in the life cycle of each intestinal cell of the larva, and that, in question of individuality of chromosomes or 'Kern-plasma Relation,' these cells conform to the laws governing other cells of the body.

One of the arguments urged against the Individuality Hypothesis has been variation in number. But certainly here, variation in number instead of arguing against the theory, seems to give added weight, as has been the case in many of the other exceptions to the rule of constant number. To be sure, we have here a wide variation in the chromosome number, ranging from [six] to a possible seventy-two, but always multiples of three—six, nine, twelve, eighteen, twenty-four, thirty-six, forty-eight. With the exception of one cell, (fig. 27 and the possible exception of fig. 23), no other number was ever found. In some individuals, the 'six series' appears, and in others the 'nine

series' is consistently present, which could be explained if we were to suppose a difference in initial rhythm between the maternal and paternal elements of each original chromosome pair. If multiple complexes were simply the result of the breaking up of chromosomes in degenerating cells, then we should expect to find, not simply the multiples of three, but any other number. Neither should we expect to find the chromosomes of uniform size, had they arisen by fragmentation.

We are led to believe, from the behavior of the chromosomes of these metamorphosing cells, that the three pairs of chromosomes which appear in the original gut cell are made up of quite distinct individuals, differing from each other to such a degree that chromatin split from one can not associate itself with that from another pair. So, all through the series of divisions which precede the final disintegration, we find the daughter chromosomes of each of the original chromosomes maintaining the size and appearance of the original structure; and moreover, we find each group of daughter chromosomes so closely associated that they behave as a single individual. Chromosome individuality, alone, can account satisfactorily for these conditions.

In the intestinal cells at least, there is no question of Boveri's Law of cell size being determined by the number of the chromosomes. A glance at the figures on plate 4 shows conclusively that no such relation exists, nor can the converse be true—that nuclear size is dependent upon amount of cytoplasm. Hertwig's idea of a definite fixed ratio existing between cytoplasm and nucleus does not hold here. It is true that the intestinal cells increase in size as they approach the time for disintegration, but this would seem to be due to the general acceleration in the metabolic processes, and to have no direct connection with size of nucleus or number of chromosomes. Cytoplasm and nucleus appear to be acting more or less independently. 'Kern-plasma-spannung' could scarcely be the cause for the division in two cells of equal size, of which one has a nucleus of twelve chromosomes and the other forty-eight. In these metamorphosing cells, at least, we must look for a cause for cell division other than the Kern-plasma Relation which is here extremely variable.

## SUMMARY AND CONCLUSION

1. During metamorphosis in *Culex pipiens*, the number of chromosomes in the cells of the pupal intestine is considerably increased.

2. Before disintegration of the cells begins, the chromosomes of each pupal gut cell pass through a number of longitudinal divisions resulting in three or four multiplications.

3. The number of chromosomes in the multiple complex is always a multiple of three—oftenest twelve, twenty-four, forty-eight; but frequently nine, eighteen, thirty-six, and even seventy-two may appear.

4. The triplex divisions of the chromosomes apparently arise through premature splitting of one member of each pair of chromosomes in the original complex or by a precocious division of one of each of the homologous elements of the bivalent chromosomes.

5. The size relation between nucleus and cytoplasm is extremely variable.

6. It appears that in the resting stage of those cells of normal size which contain multiple complexes, there must be an accelerated growth of each chromatin thread which splits into normal sized chromosomes in prophase, or else the cytoplasm of such cells must fail to divide and to grow while the chromosomes continue to do both. The former seems to be the probable explanation.

7. There is evidence of a parasynaptic union of sister chromosomes in the resting stage, followed by re-separation through longitudinal splitting in the prophase.

8. These sister chromosomes, the multiples of each member of the original complex, tend to remain together throughout the mitotic changes.

9. The individuality of the chromosomes is maintained until the cell disintegrates.

10. The disintegrated cells appear to be digested by the cells of the newly formed lining of the adult alimentary tract.



All of these facts suggest that increased metabolism of the older epithelial cells may be a means of supplying needed food material to the developing cells of the adult gut during the pupal changes. That this great increase in the amount of chromatin in cells which have attained their growth, functioned for a time, and are about to be absorbed, is not accidental, or simply a process of degeneration, seems reasonably clear from the uniformity and universality of this increase in the intestine of *Culex*. Every cell of the larval gut epithelium apparently passes through the whole series of changes above described before it reaches the stage of disintegration. If this were simply a process of degeneration, it would be hard to account for the tremendous growth in the chromatin material and for the retention of the individuality of the chromosomes to the end. One would expect the processes observed in the disintegration of the cells, to come directly without these elaborate preparatory phenomena. It would seem that we have here, not the hit-or-miss phenomena of degenerating cells, but a definite adaptation to provide for the support of the organism during metamorphosis.

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## PLATES

## PLATE 1

### EXPLANATION OF FIGURES

All drawings were made with camera lucida, Spencer homogeneous immersion lens 1.8 mm., Zeiss no. 12 compensating ocular. All, except figure 6, were then enlarged two diameters, giving a magnification of 5800. In reproduction, the drawings have been reduced one-third.

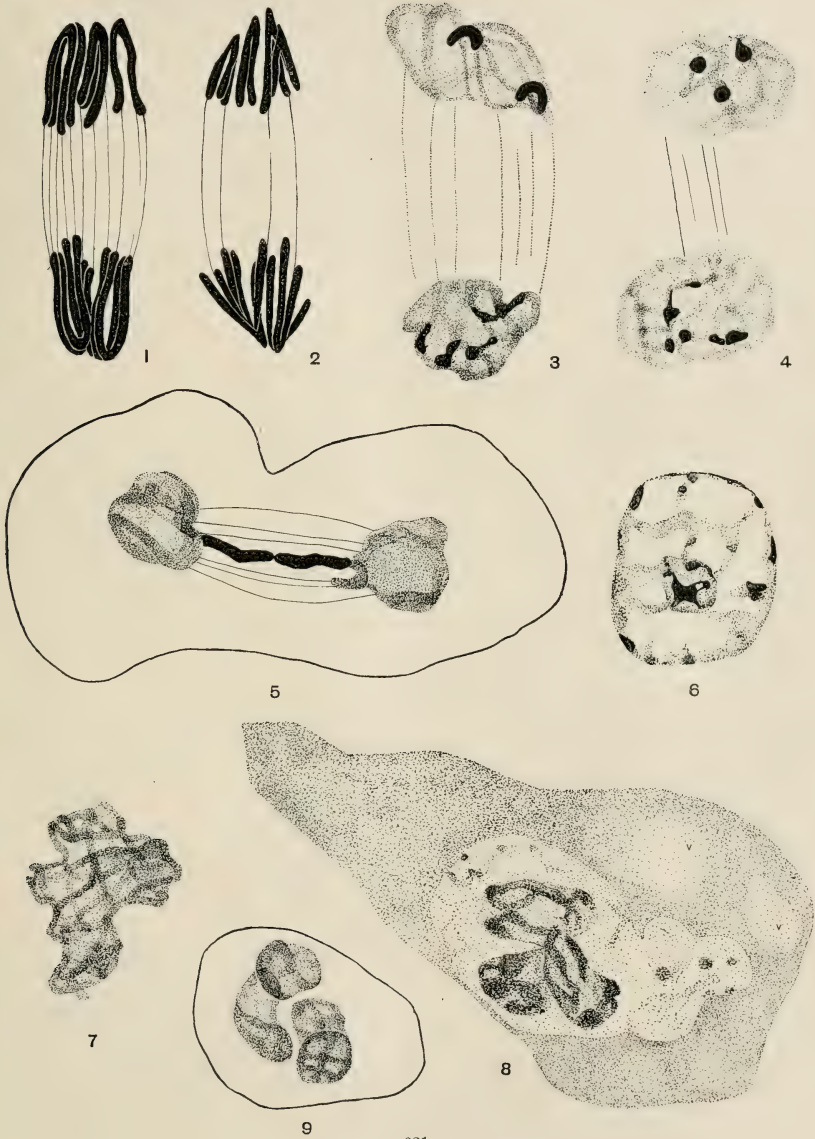
1 and 2 Late anaphases from intestinal epithelium of *Culex pipiens*, showing six pairs of chromosomes passing to each pole. Figure 1 from stomach (fig. 33, region 8). Figure 2 is from Dr. Steven's slide, position not known. Note pointed tips of *V*'s. Compare 31 from same individual.

3 and 4 Telophases from stomach (fig. 33, region 6).

5 Early telophase showing two lagging chromosomes. This cell is from the same individual as 2 and 31. Position not determined but probably stomach.

6 Resting stage from stomach (region 7) showing chromatin scattered about periphery of nucleus and collecting around the nucleolus. The magnification is one-half that of all other figures. Compare with Miss Taylor's 'clock face figure.'

7, 8, and 9 Very early prophases showing successive stages in the formation of three separate vesicles containing the chromatin threads. Figure 7, from stomach (region 6). Figure 9, from colon, (region 2). Figure 8 shows a cell about to disintegrate. Note the vacuoles, *V*, *V*, just appearing in the cytoplasm and the break in the nuclear wall.



## PLATE 2

### EXPLANATION OF FIGURES

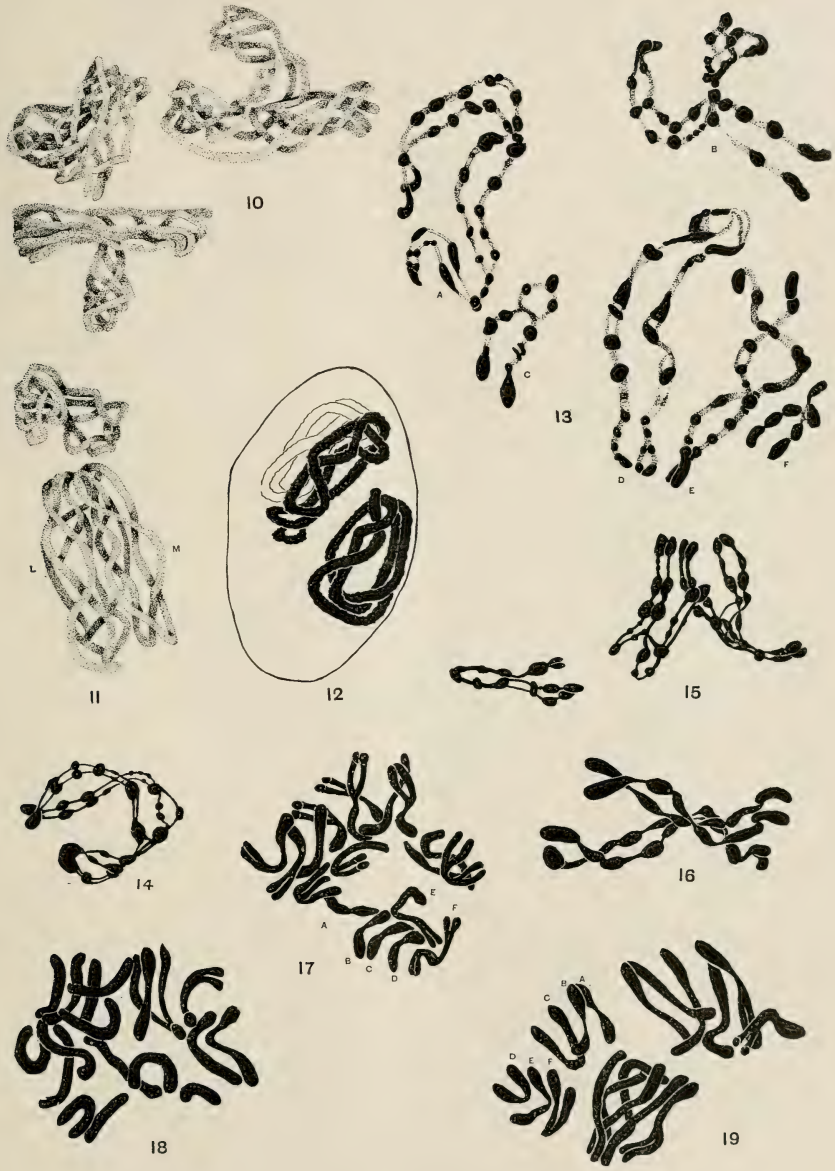
10 and 11 Early prophases from cephalic part stomach (region 10) showing threads shortening, straightening and thickening. The lower group of figure 10 shows the ends of chromosomes lying to the left. Both figures show early division of the thread. The two groups of long chromosomes, *L* and *M*, in figure 11 have straightened, while the group of short chromosomes is still folded.

12 Early prophase from ileum (region 4) showing threads in three groups surrounded by nuclear membrane.

13, 14, 15, and 16 Early prophases showing chromatin collecting in granules as the threads shorten. From stomach (region 10). Figure 13 shows two sections of the same cell, *A* and *B* in one, *C*, *D*, *E*, and *F* in the other.

17, 18, 19 Prophases. Figures 17 and 18, stomach. Figure 19 ileum. Figures 17 and 19 show three groups of six chromosomes each. The small group in each case suggests the derivation of six instead of four or eight through longitudinal division of the original short bivalent chromosome. In figure 17, *A* and *B*, *C* and *D* probably represent the completed division of two chromosomes corresponding to *E* and *F* which are still elongated and have not completed the division. In figure 19, *A* and *B*, *D* and *E*, probably represent the same condition and the larger groups in this cell also show such an early division of one chromosome of each pair. Figure 18 shows a group of eighteen chromosomes, of which two are nearly divided.





## PLATE 3

### EXPLANATION OF FIGURES

20 Prophase from stomach (region 6) showing eighteen chromosomes in three groups. The middle group shows clearly the early splitting of two of four chromosomes, *A* and *B*. The size difference indicates that the division has just taken place and the chromosomes have not had a chance to grow to normal size.

21 a, b, c, d Normal somatic metaphase complexes. Figure 21 a metaphase group from stomach epithelium showing size of chromosomes in the 'normal' gut cell. Figure 21 b, c and d, groups from nerve tissue showing typical grouping found in all somatic tissues.

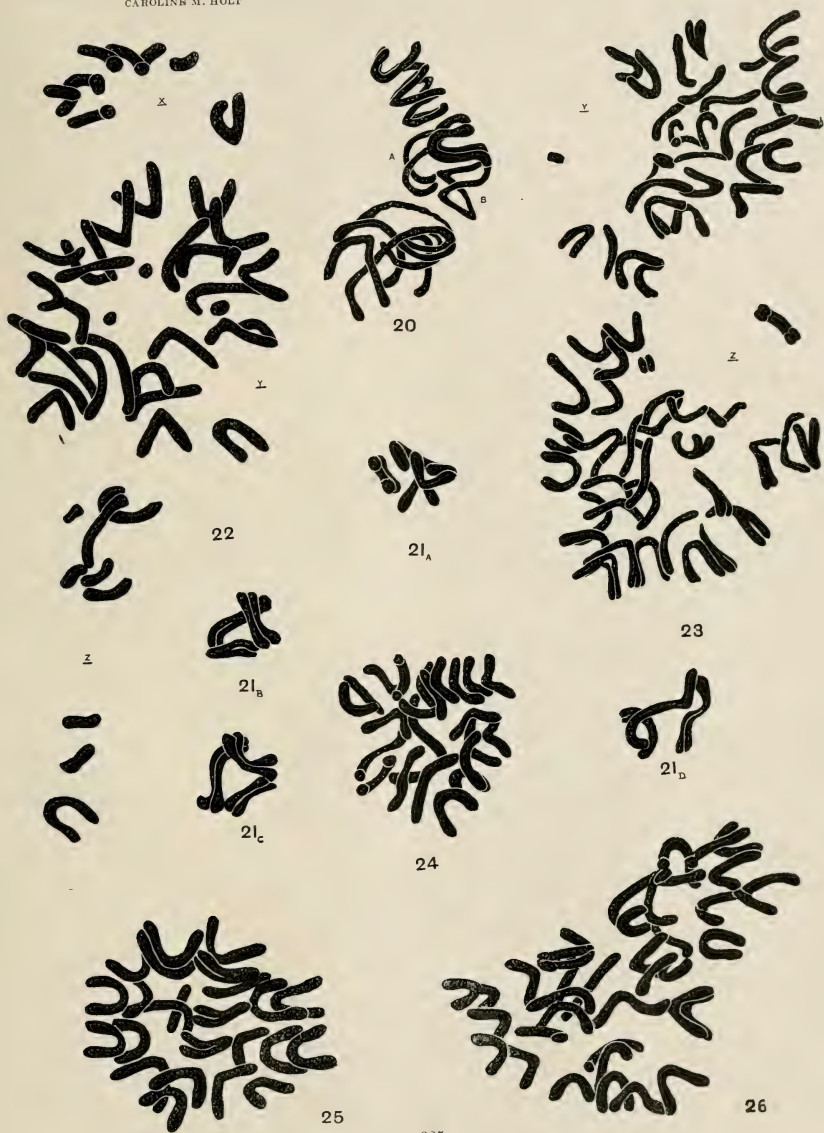
22 Metaphase group from stomach (region 6). *x*, *y* and *z* represent three sections of the same cell. When superimposed, the group is found to contain 36 chromosomes.

23 Metaphase group from ileum (region 4). *Y* and *Z* are two sections of the same cell. The number in this group is probably 72, although there appear to be but 68 chromosomes.

24 Metaphase, showing 24 chromosomes—from Dr. Stevens' slide—region undetermined.

25 Metaphase from ileum (region 3)—24 chromosomes. Three in center of group show splits.

26 Metaphase from stomach (region 6) showing 36 chromosomes, grouped in three groups of 12 each.



## PLATE 4

### EXPLANATION OF FIGURES

27 Metaphase from ileum (region 4). *G* and *H* are two sections of the same cell. When superimposed, 42 chromosomes may be counted. This number is possibly due to irregularity in time of division.

28 Metaphase from ileum (region 4). 12 chromosomes.

29 Prophase from ileum (region 5). 6 chromosomes.

30 Metaphase from ileum (region 5). 18 chromosomes, only 17 show in drawing. Figures 27, 28, 29, 30 show loss of typical size relations between cytosome and chromatin mass.

31 Anaphase from Dr. Stevens' slide. 12 pairs chromosomes. Note two lagging chromosomes.

32 Early anaphase from colon (region 2) 6 pairs chromosomes. These chromosomes seem to be smaller than those of other regions drawn.

33 Diagram of alimentary tract of pupa showing regions included in sections studied. 1, rectum; 2, colon; 3, 4, 5, ileum; 5, region of entrance of Malpighian tubules into gut; 6, 7, 8, 9, 10, stomach in abdominal region.







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